SAFETY AND PHARMACOLOGICAL PROFILE OF INJI DRAVAGAM

The dissertation Submitted by

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INTRODUCTION

INTRODUCTION

The experimental evaluation of ***INJI DRAVAGAM*** extract of *Zingiber officinale* and *Carum copticum* on wistar albino rats on both sex reveals the drug was evaluated for Anti-ulcer (peptic ulcer), Anti-inflammatory and Analgesic activities on gastric mucosa.

As per the Siddha literature "Yaakopu Vaithiya chinthamani 500" approved by concerned department HOD.

The wistar albino rats tried for Acute, Sub-acute and sub-chronic toxicity studies etc.

The extract of trial drug *Inji Dravagam* was prepared as per non shasthric preparation and approved by IAEC Ref no (KKCP/2015/28-10.08.2015)

The qualitative and quantitative analysis on *Inji Dravagam* was carried out in National Institute of Siddha and Captain Srinivasamurthi Research center for Ayurveda and Siddha drug Development in Arumbakkam, chennai.

The toxicity studies were carried out for acute, sub-acute toxicity studies at K.K college of pharmacy in Gerugampakkam and sub-chronic toxicity study was carried at National Institute of Siddha.

The studies reveals acute toxicity studies in rats indicated that *Inji Dravagam* was non-toxic and no behavioral changes were observed up to the dose level of 10ml/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

As per the OECD guidelines, WHO norms for AYUSH drug *Inji Dravagam* was analyzed and reported were tabulated.

The animal study reveals histopathological examination of organs of animals administered with highest dose showed no changes in the cellular architecture. This could be conformed no- observed-adverse-effect level (NOAEL) on acute, repeated 28days oral and 90 days oral toxicity studies.

As per the studies the drug ***Inji Dravagam*** has no remarkable toxicities in the animal models and this drug may be tried further for clinical use.

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

AIM

To evaluate the safety and pharmacological profile of the test drug "*Inji Dravagam"* in animal model.

OBJECTIVES

- Review of various information both (Siddha and modern) relevant to the study.
- Preparation of the trial drug as per classical Siddha literature.
- Analysis of the trial drug

    - Pytochemical analysis.
    - Chemical analysis to evaluate acid and basic radicals.
    - Gas Chromatography.
    - ICP-OES.

- **Toxicity study:**

    - Acute oral toxicity study (OECD - 423 Guideline)
    - 28 days Repeated oral toxicity (OECD - 407 Guideline)
    - 90 days Repeated oral toxicity (OECD - 408 Guideline)

- **Pharmacology activity in wistar albino rats and mice:**

    - Anti ulcer activity *(*Aspirin induced method)
    - Anti inflammatory activity (Cotton pellet induced granuloma method)
    - Analgesic activity (EDDy's Hot plate method)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE OF *INJI DRAVAGAM*

Drug selection

In this research work, the ***Inji Dravagam***[1] herbal formulation has been selected to evaluate as a trial drug from Siddha literature **Yaakopu Vaithiya Chinthamani-700.**

Ingredients

1. Purified fresh Ginger juice - *(Zingiber officinale)*
2. Purified Omam - *(Carum copticum)*

Source of Collection

Fresh gingers were collected from Tambaram sanatorium, Chennai, Tamilnadu. Omam was procured from a well reputed country shop in Parrys, Chennai.

Identification and Authentication of the drug

The Herbal drugs were Identified and authenticated by competent authority department of Gunapadam, National Institute of Siddha, Tambaraum sanatorium, Chennai.

Purification of Ingredient

Purification of *Omam*[2]

Omam was purified by soaked in limestone water for three hours and then it was fried.

Purification of *Inji*[3]

Inji was purified by removing the outer layer of skin.

இஞ்சி

(Zingiber officinale)

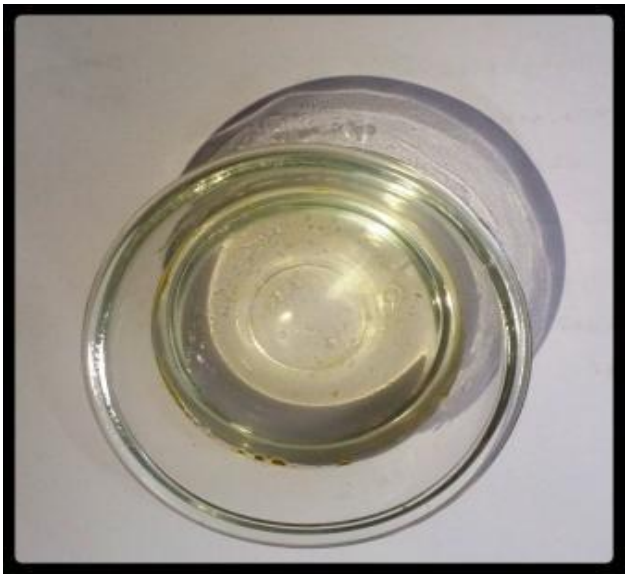
ஓமம்

(Carum copticum)

Method of drug preparation

Purified Fresh raw ginger is taken then smashed and squeezed to get the fresh juice. The fresh juice is collected in mud vessel. Along with the fresh juice, Omam is added and then subjected to the distillation process by vaaluka yanthram. Finally Dravagam was collected and stored.

The distillation process was done at Gunapadam practical lab of NIS.



INJI DRAVAGAM

Labelling

Name of the preparation	:	***Inji Dravagam***
Date of preparation	:	12/06/15 and 25/02/16
Dose	:	5ml bd
Adjuvant/Vehicle	:	Water
Indications	:	Gunmam (Peptic ulcer)
Date of expiry	:	1 year from the date of manufacture

Therapeutic Administration of drug

Form of medicine	-	Liquid (Pale yellow)
Route of administration	-	Oral
Dose	-	5ml
Vehicle	-	Water
Time of administration	-	Twice in a day

REVIEW OF LITERATURE

SIDDHA ASPECT

4. REVIEW OF LITERATURE

4.1 GUNAPADAM REVIEW

இஞ்சி – *Inji*

(Zingiber officinale)

வேறு பெயர்கள்: அல்லம், ஆர்த்திரகம், ஆத்திரகம், இலாக்கொட்டை, நறுமருப்புமதில்.

Botanical Name : *Zingiber officinale*

English name : Green ginger fresh root

Family : Zingiberaceae

பயன்படும் உறுப்பு: கிழங்கு

சுவை : கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை:

அகட்டுவாய்வகற்றி

பசித்தீத்தூண்டி

உமிழ்நீர்பெருக்கி

செரிப்புண்டாக்கி

வெப்பமுண்டாக்கி

தடிப்புண்டாக்கி

குணம்:

இஞ்சிக் கிழங்குக் கிருமல் ஐயம் ஒக்காளம்

வஞ்சிக்குஞ் சன்னிசுரம் வன்பேத - விஞ்சுகின்ற

சூலையறும் வாதம்போந் தூண்டாத தீபனமாம்

வேலையுறுங் கண்ணாய் - விளம்பு. **(அகத்தியர் குணவாகடம்)**

பொருள்:

வெள்ளோக்காளம், வளிசூலை, செரியாக் கழிச்சல் இவை போம். பசியுண்டாகும்.

வழக்கு :

இஞ்சியைப் பாலில் அரைத்துக் கழற்சியளவு 18மி.லி பாலில் காலை மாலை கொடுத்துவர குன்மம் இருமல் மயக்கம் அழல்வாய்வு தீரும். பசிஉண்டாகும்.

இஞ்சி தேனூறல் செய்து கொடுக்க அகட்டை பற்றிய வயிற்று வலி, வயிற்று பொருமல், வாந்தி, குடல் நோய்கள் தீரும்.

செரியாக் கழிச்சலுக்கு இஞ்சிச் சாற்றை, தொப்புளைச் சுற்றித் தடவலாம்.

வாந்தி, ஓக்காளம், இவைகளுக்கு இஞ்சி சாறு, வெங்காய சாறு, வகைக்கு ஒரு தோலா வீதம் எடுத்து கலந்து கொடுக்கலாம்[4].

இஞ்சி சேரும் மருந்துகள்:

- நெல்லிக்காய் லேகியம்[5]
- சௌபாக்யச் சுண்டி[6]
- இஞ்சி இரசாயனம்[7]
- இஞ்சி குழம்பு[8]

2. ஓமம்

(C*arum copticum)*

வேறு பெயர்கள்: அசமோதம், தீப்பியம்.

Botanical Name : C*arum copticum*

English Name : The Bishops weed

Family : Apiaceae

பயன்படும் உறுப்பு: விதை

சுவை : கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை :

பசித்தீத்தூண்டி

இசிவகற்றி

அகட்டுவாய்வகற்றி

அழுகலகற்றி

வெப்பமுண்டாக்கி

உரமாக்கி

உமிழ்நீர்பெருக்கி

குணம்.

சீதசுரங் காசஞ் செரியாமந் தம்பொருமல்

பேதியிரைச் சல்கடுப்பு பேராமம் -ஓதிருமல்

பல்லொடுபல் மூலம் பகமிவைநோ யென்செயுமோ

சொல்லொடுபோம் ஓமமெனச் சொல்.

பொருள்:

செரியாமந்தம், பொருமல், கழிச்சல், குடலிரைச்சல் இவை போம்.

வழக்கு:

ஓமம், சுக்கு, சித்திரமூல வேர்பட்டை இம்மூன்றும் ஓரிடை பொடித்து கலந்த எடைக்கு நேர் கடுக்காய் பொடி கலந்து கொடுக்க மந்தம் தீரும்.

ஓமம், மிளகு வகைக்கு 34கிராம் இவற்றை வெதுப்பி வெல்லம் 34கிராம் சேர்த்தரைத்து, 10 நாள் கொட்டை பாக்களவு சாப்பிடவும் வயிற்றுக்கடுப்பு, பொருமல், கழிச்சல் தீரும்.

ஓமத்தீநீர் 30-60மிலி கொடுக்க ஊழி, வயிற்றுபொருமல், வயிற்றுவலி, செரியாக்கழிச்சல், மந்தம், இவைகளை தீர்க்கும்.

ஓமம், இந்துப்பு, ஆடாதோடைச்சாறு, இஞ்சிரசம், பழச்சாறு, புதினாச்சாறு இவைகளை பாவனை செய்து கொடுக்க அசீரணம் தீரும்.

சேரும் மருந்துகள்:

- முப்பிரண்டை சூரணம்[9]
- சாமுதரச் சூரணம்[10]
- குமட்டிக்காய் குழம்பு
- அயனாதி வடகம்[11]

REVIEW OF LITERATURE

BOTANICAL ASPECT

4.2 BOTANICAL REVIEW

INJI – *Zingiber officinale*

Botanical name - *Zingiber officinale*

Family - Zingiberaceae

VERNACULAR NAME:

English	:	Ginger
Hindi	:	Adrakh
Tamil	:	Inji

PARTS USED:

Rhizome

ORGANOLEPTIC CHARACTERS

Odour	:	Aromatic
Taste	:	Pungent, Hot,

ACTION

Carminative

Stomachic

Stimulant

Digestive

Sialagogue

Rubefacient[12]

CHEMICAL CONSTITUENTS

Volatile oils

Gingerol, shogaol, zingiberene and zingiberol1-3[13]

PHYTOCHEMICAL PROPERTY

α-curcumene , β-D- curcumene, α- bergamotene , β-phellandrene, calamene, α - bisabolene, D-borneol and its acetate ,camphene, geranial, hepatane, methylhepatone, glutamic acid, gingerone[14].

MEDICINAL USES:

It is recommended during pregnancy and treating for morning sickness.

It is aromatic and beneficial in dropsy, dyspepsia, digestive and stomachic.

Ginger decotion is give for colic pain, tympanites and vomiting.

Ginger is used in tooth-ache, gout, rheumatism of the jaws.

Relaxed sore throat, hoarseness and loss of voice are benefited by chewing a piece of ginger so as to produce copious flow of saliva.

Ginger possess anti oxidant property and may be added to edible oils and fats to protect them against oxidative rancidity[15].

SUPPORTIVE ARTICLES:

Anti-ulcer activity:

Indomethacin induced ulcer - The extract used were freshly prepared as a suspension in normal saline and administered per orally to the animals in 5 ml/kg doses. Erosions formed on the glandular portions of the stomach of rat models were counted and the ulcer index calculated as described by Main and Whittle, 1975. (Urushidani et al 1979). Ginger extract dose of 100 mg/kg showed 71% inhibition of ulcer, which was comparable to that of ranitidine, the anti-ulcer drug used, which had 78% inhibition[16].

Anti-Inflammatory activity:

Ginger has been shown to share pharmacological properties with non-steroidal anti-inflammatory drugs (NSAIDs) because it suppresses prostaglandin synthesis through the inhibition of cyclooxygenase-1 and cyclooxygenase-2. However, ginger can be distinguished from NSAIDs based on its ability to suppress leukotriene biosynthesis by inhibiting 5-lipoxygenase[17]. (Mahady G.B et al, 2003)

Further related activities are Anti inflammatory, Analgesic, Antiemetic, Antiarthritic, Antioxidant[18].

OMAM - C*arum copticum*

BOTANICAL NAME - *Carum copticum*

FAMILY - Apiaceae

VERNACULAR NAME:

English : The bishops weed

Hindi : Ajvayam

Sanskrit : yavani

PART USED:

Seeds

ORGANOLEPTIC CHARACTERS:

Taste : Bitter, Pungent

Nature : Hot

Division : Pungent

ACTION:

Stomachic

Anti-spasmodic

Tonic

Anti- septic

Carminative

Aphrodisiac

Laxative[19]

CHEMICAL CONSTITUENTS:

Carum copticum have different classes of chemical constituent such as Volatile oil, Minerals and Vitamin, Steroid and other constituents.

Volatile oil:

Thymol, para-cymene, gama-terpinene are main volatile oil constituent. Carvacrol, α- and β-pinene, terpine phenolic compounds is secondary constituent[20].

Minerals and vitamins:

Minerals, Calcium, Phosphorous, Iron and Nicotinic acid[21].

Steroid:

6-O-β-glucopyranosyloxythymol,

Phytochemicals:

Fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavones[23].

MEDICINAL USES:

It is used for therapeutic effect including bloating, fatigue , diarrhoea , abdominal tumors, and respiratory distress.

It has benefits such as anti-fungal, antioxidant, anti parasitic, and hypolipidemic effect[24].

SUPPORTIVE ARTICLES:

Indomethacin-induced gastric ulcer

Indomethacin was suspended in 1% carboxymethylcellulose in water (6 mg/ml) and administered to the fasted rats in a dose of 30mg/kg (0.5 ml/100 g). Rats were treated with ajowan suspension(250 and 500 mg/kg, orally) 30 min before indomathacin. Control rats were treated similarly with an equivalent amount of vehicle (Bhargava et al., 1973). The stomachs of the animals shows significant reduction of gastric ulceration was noted[25].

Thymol also possesses **anti-inflammatory**, **analgesic** and **local anesthetic** effects.

PHARMACEUTICAL REVIEW

5. PHARMACEUTICAL REVIEW

Dravagam or Theeneer is the distilled essence, which contains the volatile constituents of the drugs used in the preparation, in a medium of water and is equivalent to " aqua" or water of the western pharmacopoeia.

The term Dravagam or Theeneer denotes the acceptable aromatic nature of the drug and indicates that it is in the Aquous state.

Preparation

The preparation of Dravagam or Theeneer involves the efficient distillation of water soaked coarse powders of the drugs. The volatile principles which are evolved admixture with water vapor from the still are condensed and taken.

Equipment required

Bottle of large capacity of handle the distillate.

An assembly of apparatus as described below or modifications thereof for distillation

Process of preparation

The drugs are crushed into a coarse powder and soaked in the prescribed quantity of water, for about a day or at least over night and then charged into the still, along with the water.

The lid is tightly set in and around to prevent leakage of vapours. For the purpose of sealing a cloth ribbon with a paste of black gram is used. Heat is applied to the drug mixture and distillate is collected in bottles, and then the whole condensate from one charging is thoroughly mixed, because the portions that had condensed later would be a weaker in concentration. A continuous water current should be maintained in the condenser.

Storage

Dravagam or theeneer should be stored in air tight containers of glass. If kept open, the volatile active principles will be lost by evaporation.

Dravagam or Theeneer is administered along with an equal volume of water[26].

ANALYTICAL STUDY

CHEMICAL ANALYSIS

6. ANALYTICAL STUDY OF THE *INJI DRAVAGAM*

Analytical study of the trial drug brings the validation to be used as a medicine by subjecting the trial drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its organoleptic charcterization, physical characteristic, phytochemical property and also to assess the active principles and elements present in the trial drug. Thus analytical study brings the efficacy and potency of the drug.

As per AYUSH protocol for analytical study, the following parameters were evaluated.

- **Organoleptic character**
  - Colour
  - Odour
  - Taste
- **Physicochemical analysis**
  - Physical characterization
- **Chemical analysis**
  - Primary acid and basic radicals
- **Phytochemical analysis**
  - TLC and HPTLC
- **Elemental analysis**
  - ICP-OES
- **Gas chromatography**

6.1 ORGANOLEPTIC CHARACTER

Colour

The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

6.3. CHEMICAL ANALYSIS OF INJI DRAVAGAM

The chemical analysis of *INJI DRAVAGAM* was carried out in Bio chemistry Lab, National institute of siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Colourless	
2.	**Test for Silicate** 2ml of the sample was shaken well with distilled water.	No Sparingly soluble	Absence of Silicate
3.	**Action of Heat:** 2ml of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	**Flame Test:** 2ml of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame	Absence of copper
5.	**Ash Test:** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Appearance of yellow colour flame	Absence of sodium

Preparation of Extract:

5ml of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

S.N	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No cloudy appearance	Absence of Sulphate
2.	**Test For Chloride:** 2ml of the above prepared extracts was added with 2ml of dil-HCl was added until the effervescence ceases off.	Cloudy appearance present	**Presence of Chloride**
3.	**Test For Phosphate:** 2ml of the extract were treated with 2ml of dil. Ammonium molybdate solution and 2ml of con.HNo3	No cloudy yellow appearance present	Absence of Phosphate
4.	**Test For Carbonate:** 2ml of the extract was treated with 2mldil. magnesium sulphate solution	No cloudy appearance	Absence of carbonate
5.	**Test For Nitrate:** 1ml of the extract was heated with copper turning and concentrated H_2So_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test For Sulphide:** 1ml of the extract was treated with 2ml of con. HCL	No rotten egg smelling gas was evolved	Absence of sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate

8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil. acetic acid and 2 drops of dil. Benzidine solution were added.	No characteristic changes	Absence of nitrite
9.	**Test For Borate:** 2ml of the extract was made into paste by using dil-sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green colour	Absence of borate
II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil-potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper:** 2ml of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour precipitate	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil-sodium hydroxide was added in 5 drops.	No characteristic changes	Absence of Aluminium.
4.	**Test For Iron:** a. To the 2ml of extract add 2ml of dil-ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	No Red colour appeared	Absence of Iron
5.	**Test For Zinc:** To 2ml of the extract dil-sodium hydroxide solution was added in 5 drops and dil-ammonium chloride is added.	No White precipitate was formed	Absence of Zinc

6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil-ammonium oxalate solution	Cloudy appearance and white precipitate formed	**presence of calcium**
7.	**Test For Magnesium:** To 2ml of extract dil-sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil-sodium hydroxide solution are added.	Mild Brown colour appeared	**Presence of ammonium**
9.	**Test For Potassium:** 2ml of extract was treated of with 2ml of dil-sodium nitrite solution and then treated with 2ml of dil-cobalt nitrate in 30% dil-glacial acetic acid.	No Yellow precipitate was obtained	Absence of potassium
10.	**Test For Sodium**: 2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil-sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil-sodium hydroxide solution.	No Brownish red precipitate is obtained	Absence of arsenic
III. Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil-Iodine solution	No Blue colour developed	Absence of starch

2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and boil for 2 minutes and added 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No Brick red colour was developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the extract was treated with 2ml of dil-potassiumlodide solution. b) 2ml of the extract was treated with 2ml of dil-picric acid. c) 2ml of the extract was treated with 2ml of dil-phosphotungstic acid.	Yellow colour developed	**Presence of Alkaloids**
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil-ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil-Potassium permanganate solution is added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No violet colour	Absence of amino acid
7.	**Test For Type of Compound:** 2ml of the extract was treated with 2 ml of dil-ferric chloride solution.	No green and red colour No Violet colour developed No Blue colour developed.	Absence of quinolepinephrine pyrocatechoantipyrine Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydrouinone are present.

TLC/HPTLC finger print analysis:

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent. The Rf formula is

Rf = distance traveled by sample /distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions.

The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value[27]

RESULTS:

The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996

TLC and HPTLC Methodology:

Taken 15ml of the sample add 60ml Ethyl acetate keep it over night. Then Ethyl acetate layer was separated and dried over sodium sulphate anhydrous. Filtered and concentrated to 10ml at room temperature. 20µl, 25µl of the above solution were applied on Merck Aluminum plate pre-coated with silica gel $60F_{254}$ of 0.2mm thickness using ATS-IV. The plate was developed in *Toluene: Ethyl acetate* (8.2). The plate was dried and visualized in UV 254 and UV366nm and photographs were taken, the plate was scanned at 254nm before dipping. Then the plate was dipped in vanillin-sulphuric acid and heated at 105ºC till the colour of the spots appeared and photos were taken[28].

HPTLC has been done at captain srinivasamurthir research institute for ayurveda and Siddha drug development

ELEMENTAL ANALYSIS

D. INDUCTIVELY COUPLED PLASMA OPTICAL EMISSIOS SPECTROMETRY

The ICP- OES is a trace-level elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

Introduction

The element composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metallolids at trace, minor and major concentration.

Principle

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer, so that intension of the individual wavelength can be measured. The number of photos emitted is directly proportional to the concentration of the element. The photos may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wave length and after performing the calibration using known standards.

Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e, how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

Sample preparation – Microwave Digestion

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or sulphuric acid such that no piece of sample sticks on the slide.
- Mix thoroughly and allow reacting for few minutes.

- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes. Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made upto 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

ICP-OES has been done at SAIF,IITM, Chennai-96.

GAS CHROMOTOGRPHY : (GC)

Gas chromatography is a chromatographic separation technique based on the difference in the distribution of species between two non- miscible phase in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives, which are volatilized under the temperatures employed.

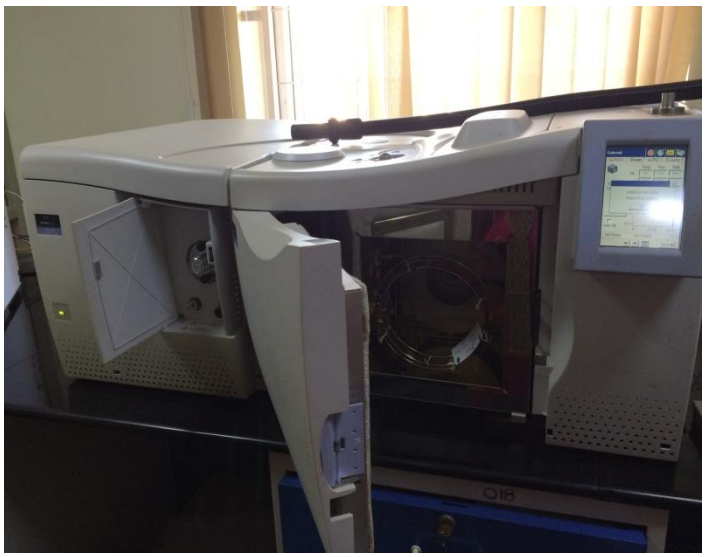
GC is based on mechanism of adsorption, mass distribution or size exclusion.

METHOD

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution (S) and the reference solution (S) as prescribed. The solution must be free from solid particles.

GC-MS SPECTROMETER

The JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI) .



GC-MS has been done at SAIF,IITM, Chennai-96.

Applications

1. Structural elucidation of organic compounds.
2. Mechanistic study of fragmentation process under mass spectrometric condition.
3. Molar mass and structural analysis of small biomolecules.

GC profile:

Instrument name	:	JEOL GC MATE II
Front inet temp	:	220 degree c
Column	:	HP 5 Ms
Carrier gas	:	high pure helium
Flow rate	:	1 ml /min
Oven temp	:	50 to 250 @ 10 deg / min
Ion chamber tem	:	250 deg c
GC interface temp	:	250 deg c
Mass analyzer	:	quadrupole with double focusing mass analyzer,
Detector	:	Photon multiplier tube
Scam	:	50 to 600 amu 70ev electron impact ionization

GC-MS has been done at SAIF,IITM, Chennai-96.

TOXICITY STUDIES

7. TOXICITY STUDIES

ACUTE ORAL TOXICITY-EXPERIMENT PROCEDURE

Acute toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 100-150 gm, were selected and oral administration of the single doses of *Inji Dravagam* was done aseptically by suspending in 1ml water.

Experimental animals:

Albino rats (wistar rats) of either sex, weighing (100-150 gm) were procured from animal housing facility, K.K college of pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature 24□□ C±1□ C and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum. They were acclimatized to laboratory condition for seven days before commencement of the experiment.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals **(KKCP/2015/028)**. Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

I. Acute oral toxicity study:

The Acute toxicity studies were performed in accordance with the OECD 423 guidelines. Female wistar rats weighing100 -150gm were selected and divided into 3 groups containing three animals in each group. The single dose of *Inji Dravagam* the starting from 1ml/kg upto 10ml/kg (1, 5, 10ml/kg) was administered orally. The drug treated animals were carefully observed individually for the toxicity signs and mortality.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements were recorded. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors were noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Test Substance	:	*Inji Dravagam*
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Male and Female Wistar Albino Rats
Age	:	More than 8 weeks
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior to and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking on fur.
Diet	:	Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	:	Potable water in polypropylene bottles *ad libitum.*
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.

OBSERVATIONS:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total period of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed.

All observations were systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern. Attention was directed to

observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed.

When animals are killed for humane reasons or found dead, the time of death should was recorded. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study[29].

Acute toxicity study has been done at K.K College of Pharmacy

REPEATED DOSE 28 DAYS ORAL TOXICITY

II.SUB ACUTE TOXICITY STUDIES of " *Inji Dravagam* " in rat - (OECD – 407 guidelines)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Inji Dravagam* was administered to rats at the dose of 5ml /kg/day and 10ml/kg/day for 28 days. The animals were observed daily for gross behavioural changes and other sign of sub acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight, each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Test Substance	:	*Inji Dravagam*
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Male and Female Wistar Albino Rats
Age	:	More than 8 weeks
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior to and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking on fur.
Diet	:	Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	:	Potable water in polypropylene bottles *ad libitum.*
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.

Justification for Dose Selection:

The results of acute toxicity studies in rats indicated that *Inji Dravagam* was non toxic and no behavioral change was observed up to the dose level of 10ml/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

As per OECD guideline three dose level were selected for the study. They are low dose (X), mid dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (10ml) body surface area of the rat (0.018) i.e X dose 0.9ml/kg, 5X dose is 5ml/kg, 10Xdose 10ml/kg.

Preparation and administration of dose:

Inji Dravagam two doses level 5ml/kg and 10ml/kg respectively were prepared. The test substance was freshly prepared every day for 28 days. The control animals were administered water 10ml/kg vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

Ten Rats (Five Male and Five Female) in each group randomly divided into three groups for dosing upto 28 days. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

i)Body Weight:

Weight of each rat was recorded on day 0 at +weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated. (table -9)

ii) Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups. (table -11)

iii) Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

iv) Mortality:

All animals were observed twice daily for mortality during entire course of study.

v) Laboratory investigation:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm3) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels by using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase

(GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 10ml /kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included heart, kidneys, liver ,spleen and pancreas of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way Anova Followed by Dunnet't' test using a computer software programme[30]. (Graph Pad Prism 5.0)

REPEATED DOSE 90 DAYS ORAL TOXICITY

7.3 90-DAYS REPEATED DOSE ORAL TOXICITY STUDY OF *INJI DRAVAGAM* (OECD GUIDELINE - 408)

Test Substance	**:** *INJI DRAVAGAM*
Animal Source	**:** King Institute, Guindy, Chennai.
Animals	**:** Wistar Albino Rats (Male -5, and Female-5)
Age	**:** 6-8 weeks
Body Weight	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard potable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** Between 22ºC $\pm$3ºC.
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**: 90 Days.**

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

90days Chronic toxicity study were carried out according to OECD 408 and were divided into 4 group. Each group of 6 animals (Male-3 and Female-3) totally 24 animals. Group I treated as a control and other three groups were treated with test drug (low dose, mid dose, high) dose for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved Number**: NIS/IAEC-I/2016/03**. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose (5X), high dose (10X). X was calculated by multiplying the therapeutic dose (2000mg) and the body surface area of the rat (0.018). i.e X dose was 0.9ml/kg. 5X dose was 5ml/kg, 10Xdose was 10ml/kg.

Preparation and Administration of Dose:

Inji Dravagam was suspended in water to obtain concentrations of 10ml/kg. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

- **Body Weight:**

  Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.

- **Clinical signs**:

  All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

- **Mortality:**

  All animals were observed twice daily for mortality during entire course of study.

- **Laboratory Investigations:**

  Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium

heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

❖ **Haematological Investigations:**

Haematological parameters were determined using Haematology analyzer.

❖ **Biochemical Investigations:**

Biochemical parameters were determined using auto-analyzer.

❖ **Histopathology:**

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technic on and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

❖ **Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't'test using a computer software programme[31] -(Graph Pad Prism5)

Sub chronic toxicity study has been done at NIS.

PHARMACOLOGICAL STUDIES

PHARMACOLOGY STUDIES

ANTIULCER ACTIVITY ON GASTRIC MUCOSA (Aspirin Induced Gastric Ulcers)

Aim:

To evaluate the antiulcer activity of *Inji Dravagam by* Aspirin Induced Gastric Ulcers.

Selection of Experimental animals:

Healthy Wistar albino rats of either sex weighing (150-250 gms) were used for this study. The animals were obtained from animal house, K.Kcollege of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2c and relative humidity of 30-70%. At 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (KKCP/2015/028) of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

Aspirin Induced Gastric Ulcers

Principle:

Aspirin was a NSAID which inhibit the synthesis of prostaglandins. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions. Aspirin also inhibit the gastric peroxidase and may increase mucosal hydrogen peroxide and hydroxyl ions level to cause oxidative mucosal damage.

Procedure:

Albino rats of either sex weighing between 150-250 gms were divided into five groups and each consisting of six rats.

Group I : Vehicle control (2ml/kg p.o.)
Group II : Negative control received only Aspirin (500mg/kg)
Group III : Standard drug Ranitidine + Aspirin (500 mg/kg)
Group IV : Received *Inji Dravagam* 1ml/kg + Aspirin (500 mg/kg)
Group V : Received *Inji Dravagam* 2ml/kg + Aspirin (500 mg/kg)

The animals were fasted for 24 hours. The test drug in varying concentration Based on the design of the experiment is administered orally 30 minute prior to aspirin at Dose of 500 mg/kg. 4 hours later the rats were sacrified by using anaesthetic ether and their Stomach were dissected and they were opened along the greater curvature for the determination of gastric lesions . Ulcer index was calculated by nothing the number of ulcers per animal and severity scored by observing the ulcers microscopically with the help of 10X lens.

Evaluation of parameters:

Effect of Free Acidity and Total Acidity:

The free acidity and total acidity was determined based on the titre values. One ml of gastric juice was pipette into 100 ml of conical flask and titrates with 0.01N NaOH using topfers reagent as an indicator (It is Dimethyl –amino-azo-benzene with phenolphthalein and used for the detection and estimation of hydrochloric acid and total acidity in gastric fluids) titrate to end point, when the solution turns to orange colour, note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the volume of NaOH which corresponds to the free acidity. Acidity (mEq/L/100g)can be expressed as

$$\text{Acidity}= \frac{volume\ of\ NaoH \times Normality\ of\ NaoH}{0.1} \times 100$$

Ulcer index:

The ulcer index was calculated by taking the mean ulcer score of each groups. Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of groups

Collection of Gastric Juice

The stomach was excised carefully opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min, the volume of the supernatant was expressed as ml /100 gm body weight. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

Ulcer Scoring

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe under 10X magnification for ulcer. Score the ulcers as below.

0 = normal coloured stomach

0.5 = red colouration

1 = spot ulcers

1.5 = haemorrhagic streaks

2 = Ulcers ≥ 3 but ≤ 5

3 = Ulcers >5

Mean ulcer score for each animal is expressed as Ulcer Index.

ANTI- INFLAMMMATORY ACTIVITY OF INJI DRAVAGAM ON WISTAR ALBINO RATS (Cotton pellet granuloma method)

AIM:

To evaluate the anti - inflammatory activity of *Inji Dravagam* in Wistar albino rats by Cotton pellet granuloma method.

Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No: KKCP/2015/028). Wistar albino rats (150- 180 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2c and relative humidity of 30-70% at 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

Experimental Design for Cotton pellet granuloma model

The animals were divided into four groups each group consists of 6 animals.

Group-I : Control - Vehicle control received distilled water (dose: 10 ml/kg).
Group-II : Standard drug - Animals treated with Dexamethasone (dose: 0.5 mg/kg).
Group-III : Animals treated with *Inji Dravagam* (1 ml/kg)
Group-IV : Animals treated with *Inji Dravagam* (2 ml/kg)

Experimental procedure

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps, subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat. After recovering

from anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg, low dose (1ml/kg) and high dose (2ml/kg) of *Inji Dravagam* for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 600C for 24 hrs. The percentage inhibition of wet weight and dry weight of the granuloma were calculated and compared.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control - Treated}}{\text{Control}} \text{ X } 100$$

ANALGESIC ACTIVITY OF INJI DRAVAGAM ON SWISS ALBINO MICE (Eddy's Hot plate method)

AIM:

To evaluate the Analgesic activity of *Inji Dravagam* in Swiss albino mice by Eddy's Hot plate method.

Selection of Experimental animals:

Healthy Swiss albino mice of either sex weighing (20-25gms) were used for this study. The animals were obtained from animal house, k.k college of pharmacy, gerugambakkam, Chennai. Animals were housed at a temperature of 24±2c and relative humidity of 30-70%. At 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (IAEC approval No: KKCP/2015/028) of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

Evaluation of Analgesic activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

Eddy's Hot plate method:

Principle:

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test is a test of the pain response in animals. It is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.

They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

Animals

Mice 20-25 g were grouped in three groups, six animals in each group.

Grouping:

Group I : **Control** - distilled water (10ml/kg, p.o.),

Group II : **Standard drug -** Pentazocine (5mg/kg, p.o.)

Group III : **Received** *Inji Dravagam* ID (1ml/kg)

Group IV : **Received** *Inji Dravagam* ID (2ml/kg)

Equipment:

Eddy's Hot plate

Procedure:

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1□ C. Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30 , 60 ,90 ,120 and 150 mins interval after drug administration.

Statistical analysis

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied. P value less than 0.05 ($P<0.05$) was considered as statistically significant.

RESULTS

RESULTS

In My research, studies like Organoleptic characters, physical characters, chemical analysis, phytochemical analysis, ICP-OES, Gas chromatography, Toxicity and pharmacological studies have been carried to know the potency and efficacy of the trial drug *Inji Dravagam*.

- Botanical aspect explains the active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating gunmam (peptic ulcer)
- The pharmacological review explains about the Evaluation of Anti-ulcer, Anti-inflammatory and analgesic activity.

STANDARDIZATION OF THE TEST DRUG

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physicochemical and Phytochemical analysis, and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Inji Dravagam.*

6.1 ORGANOLEPTIC CHARACTER

Table 1: Organoleptic character of *Inji Dravagam*

S. No	Parameters	Results
1.	**Colour**	**Pale yellow**
2.	**Odour**	**Aromatic odour**
3.	**Taste**	**Pungent**
4.	**State of matter**	**Liquid**

6.2 PHYSICAL CHARACTER

Table 2: physical character of *Inji Dravagam*

S.No.	Parameters	Result
1	**PH**	**5.02**
2	**Specific Gravity**	**0.9981**
3	**Volatile matter**	**Negligible**
4	**TLC**	**Separate 3 Nos of sheets were attached**
5	**HPTLC**	

6.3 CHEMICAL ANALYSIS

Table 3: Results of Acid radicals studies of *Inji Dravagam*

S.NO	Parameter	Observation	Result
1	Test for Sulphate	-	Negative
2	Test for Chloride	Cloudy appearance present	**Positive**
3	Test For Phosphate	-	Negative
4	Test For Carbonate	-	Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluoride &oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Interpretation

The acidic radicals test shows the presence of **Chloride**

Table 4: Results of basic radicals studies of *Inji Dravagam*

S.NO	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium .	-	Negative
4	Test For Iron .	-	Negative
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate obtained	**Positive**
7	Test For Magnesium	-	Negative
8	Test For Ammonium	Mild brown colour appears.	**Positive**
9	Test For Potassium	-	Negative
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation

The basic radical test shows the presence of **Ammonium, calcium** and absence of heavy metals such as lead, arsenic and mercury.

6.4 TLC and HPTLC analysis of *Inji Dravagam*

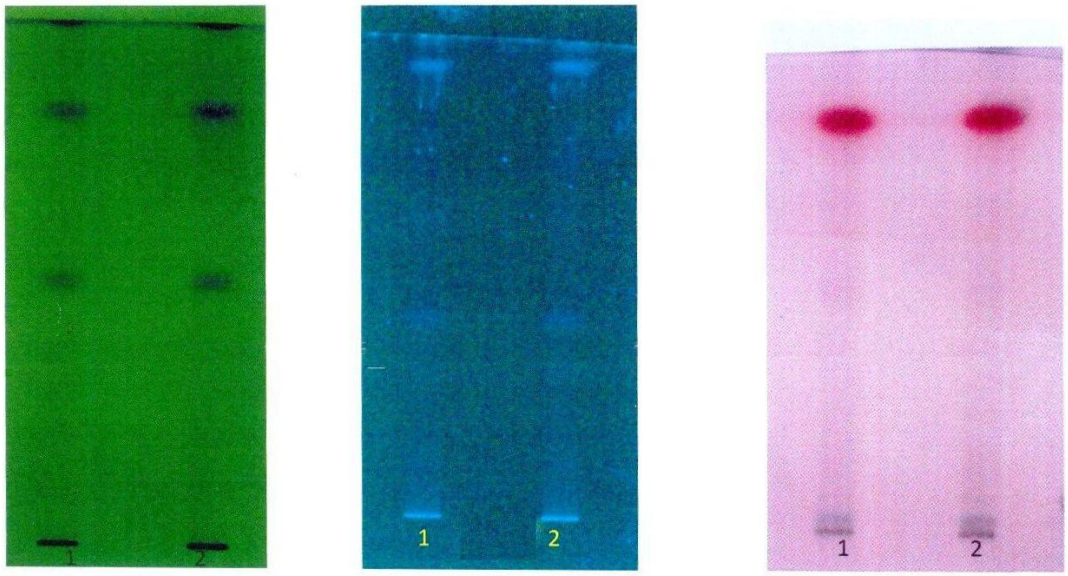
The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996

Table: 5 TLC and HPTLC analysis

S.No	254nm		366nm		Dipped in Vanillin-Sulphuric Acid	
	Colour	**Rf**	**Colour**	**Rf**	**Colour**	**Rf**
1	Green	0.56	Blue	3.0	Grey	0.04
2	Green	0.84	Blue	6.8	Grey	0.1
3	Green	099			Grey	0.53
4	Green				Grey	0.89

TLC PHOTODOCUMENTATION OF DTL SAMPLE 1510357

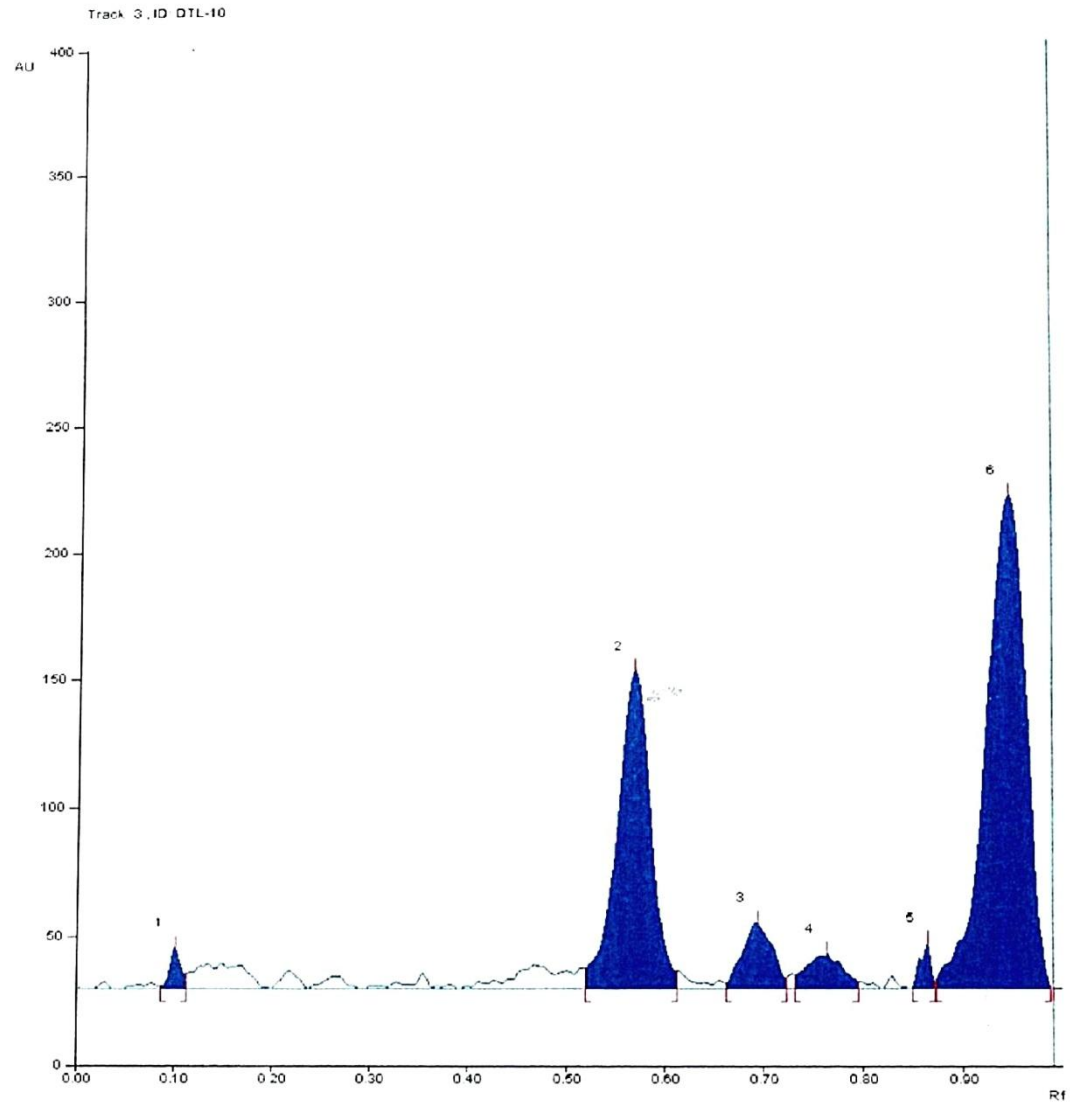
UV254nm **UV366nm** **DERIVATISEDM WITH VANILLIN SULPHURIC ACID**



Track 1 -20 µl , Track -2 -25 µl

SOLVENT SYSTEM : TOLUENE : ETHYL ACETATE (8:2)

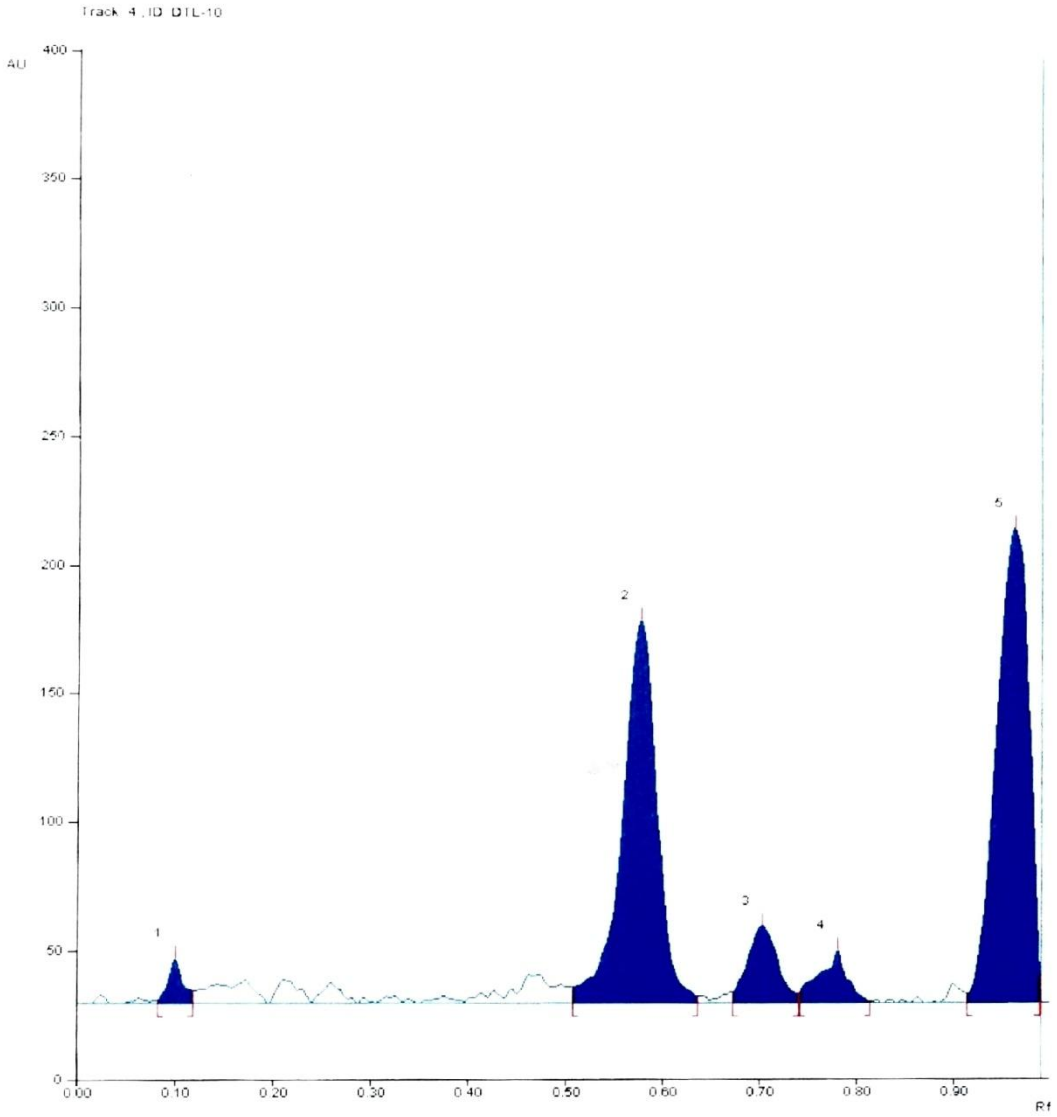
HPTLC FINGERPRINT PROFILE OF DTL SAMPLE 1510357 AT 254 nm at 20µl



Track 3-ID DTL 10

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.09 Rf	0.9 AU	0.10 Rf	15.7 AU	4.10 %	0.11 Rf	5.7 AU	152.1 AU	1.40 %	unknown *
2	0.52 Rf	7.7 AU	0.57 Rf	122.6 AU	31.91 %	0.61 Rf	6.9 AU	3418.5 AU	31.45 %	unknown *
3	0.66 Rf	2.0 AU	0.69 Rf	24.9 AU	6.50 %	0.72 Rf	4.2 AU	616.0 AU	5.67 %	unknown *
4	0.73 Rf	5.3 AU	0.76 Rf	13.0 AU	3.38 %	0.80 Rf	2.8 AU	394.7 AU	3.63 %	unknown *
5	0.85 Rf	0.2 AU	0.87 Rf	17.2 AU	4.49 %	0.87 Rf	2.7 AU	152.0 AU	1.40 %	unknown *
6	0.87 Rf	3.3 AU	0.95 Rf	190.6 AU	49.63 %	0.99 Rf	0.7 AU	6135.2 AU	56.45 %	unknown *

HPTLC FINGERPRINT PROFILE OF DTL SAMPLE 1510357 AT 254 nm at 25µl



Track 4 ID DTL-10

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	1.1 AU	0.10 Rf	17.0 AU	4.22 %	0.12 Rf	4.9 AU	215.1 AU	1.98 %	unknown *
2	0.51 Rf	6.0 AU	0.58 Rf	149.2 AU	37.09 %	0.64 Rf	2.6 AU	4371.6 AU	40.25 %	unknown *
3	0.67 Rf	4.2 AU	0.70 Rf	30.0 AU	7.45 %	0.74 Rf	3.6 AU	747.2 AU	6.88 %	unknown *
4	0.74 Rf	4.0 AU	0.78 Rf	20.4 AU	5.07 %	0.81 Rf	0.5 AU	466.0 AU	4.29 %	unknown *
5	0.91 Rf	3.6 AU	0.96 Rf	185.7 AU	46.18 %	0.99 Rf	10.5	061.1 AU	46.60 %	unknown *

6.5 ELEMENTAL ANALYSIS

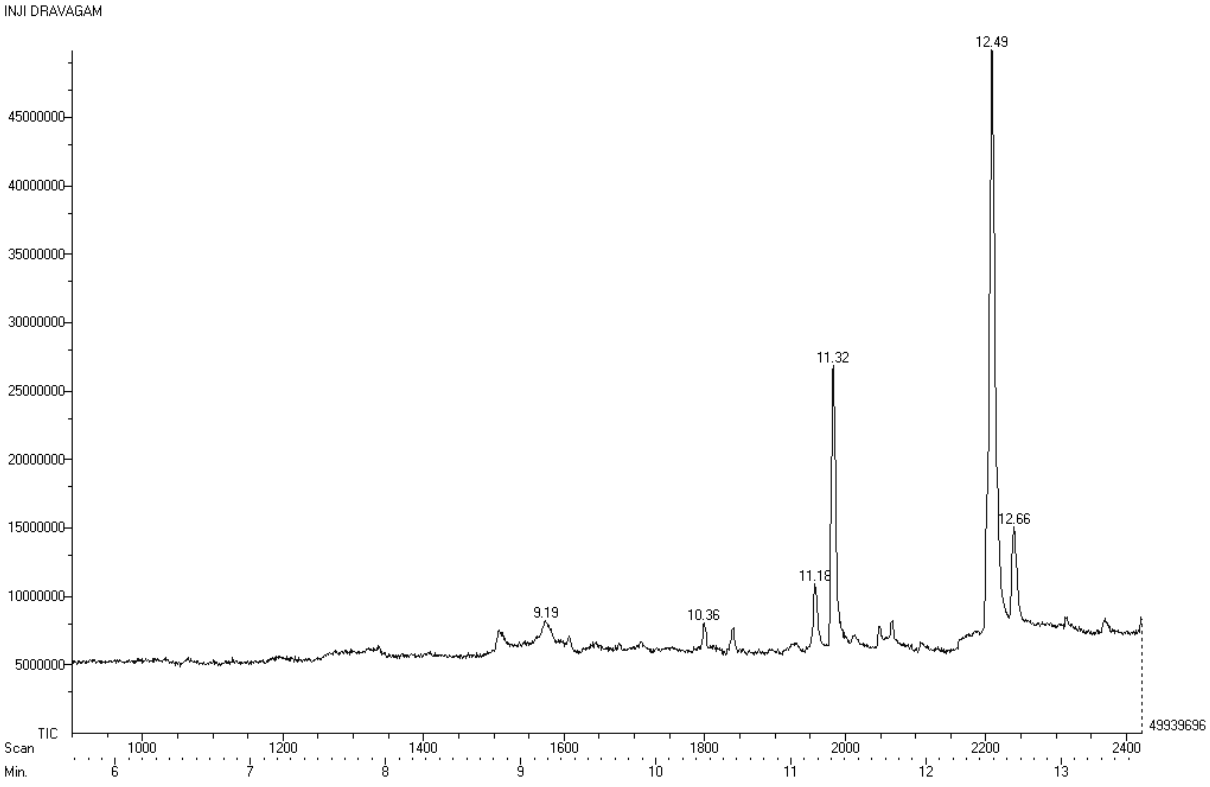
Table :6 ICP-OES study results of *Inji Dravagam*

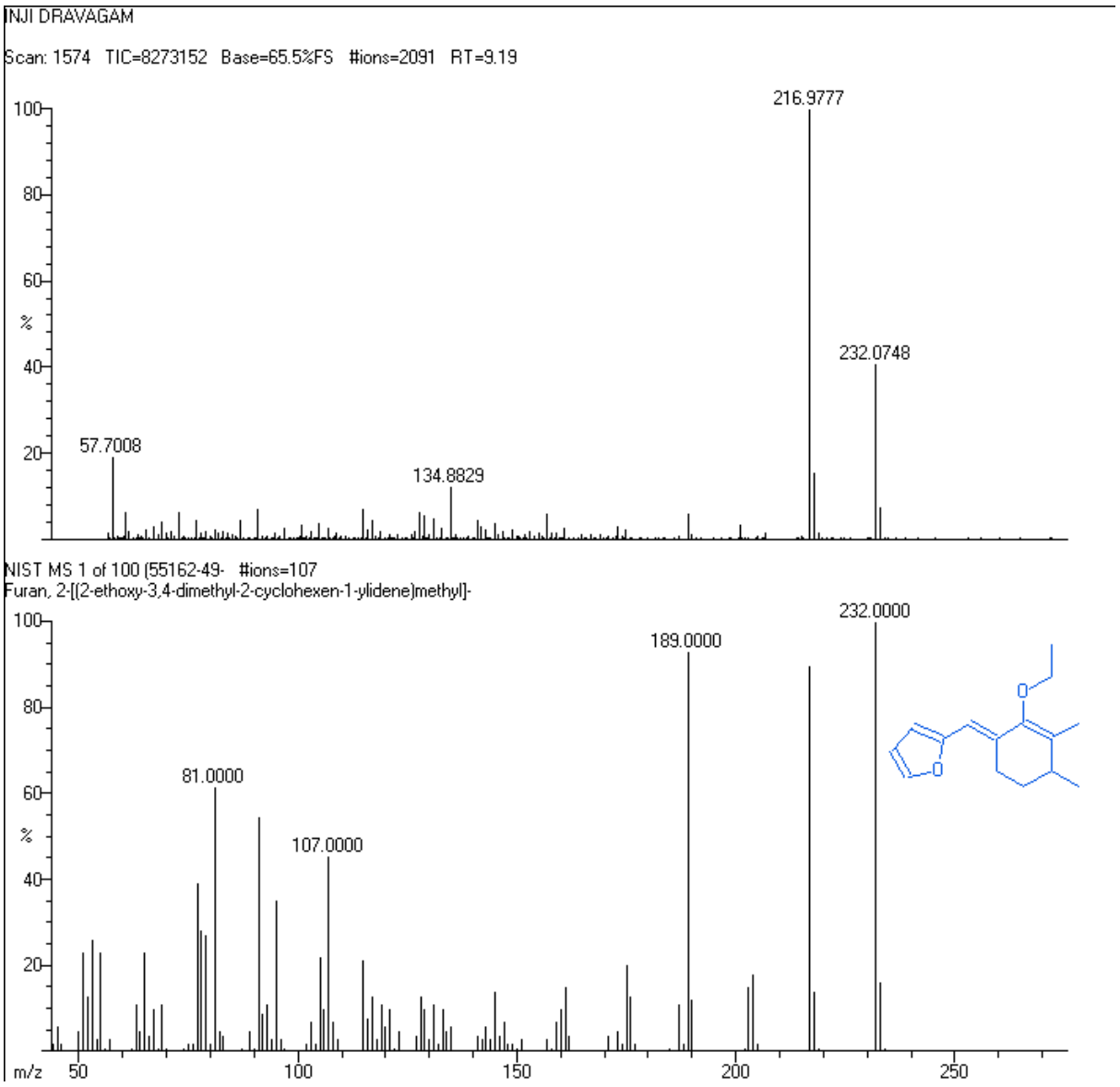
S.NO	Elements	Wavelength in nm	Inji Dravagam mg/L
1.	Arsenic	As 188.979	BDL
2.	Calcium	Ca 315.807	12.760mg/L
3.	Cadmium	Cd 228.802	BDL
4.	Copper	Cu 327.393	BDL
5.	Iron	Fe 238.204	08.346mg/L
6.	Mercury	Hg 253.653	BDL
7.	Potassium	K 766.491	23.821mg/L
8.	Magnesium	Mg 285.213	11.153mg/L
9.	Nickel	Ni 231.604	BDL
10.	Phosphorus	P 213.617	19.341
11.	Lead	Pb 220.353	BDL
12.	Zinc	Zn 206.200	01.258mg/L

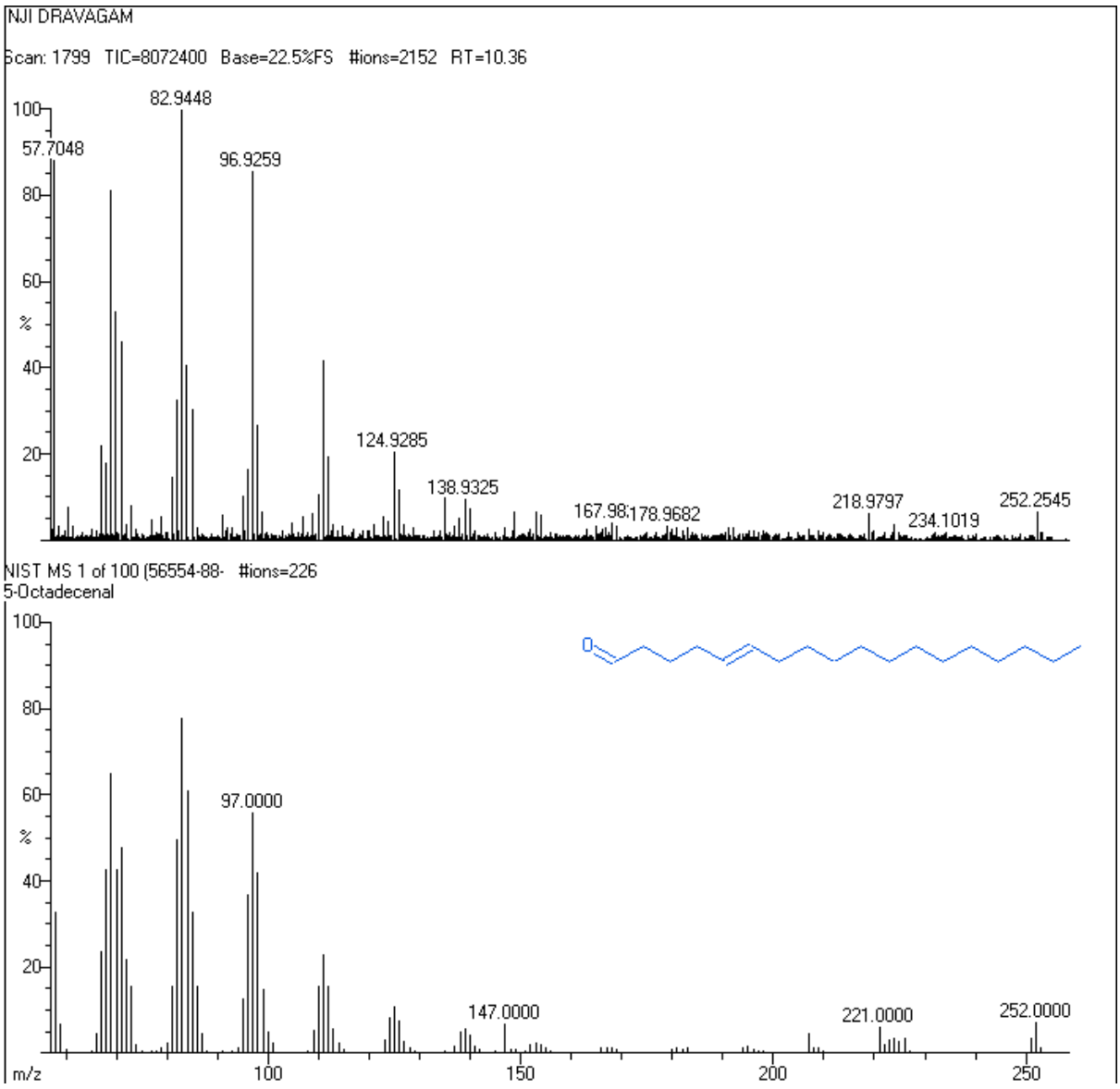
* BDL – Below Detection Limit

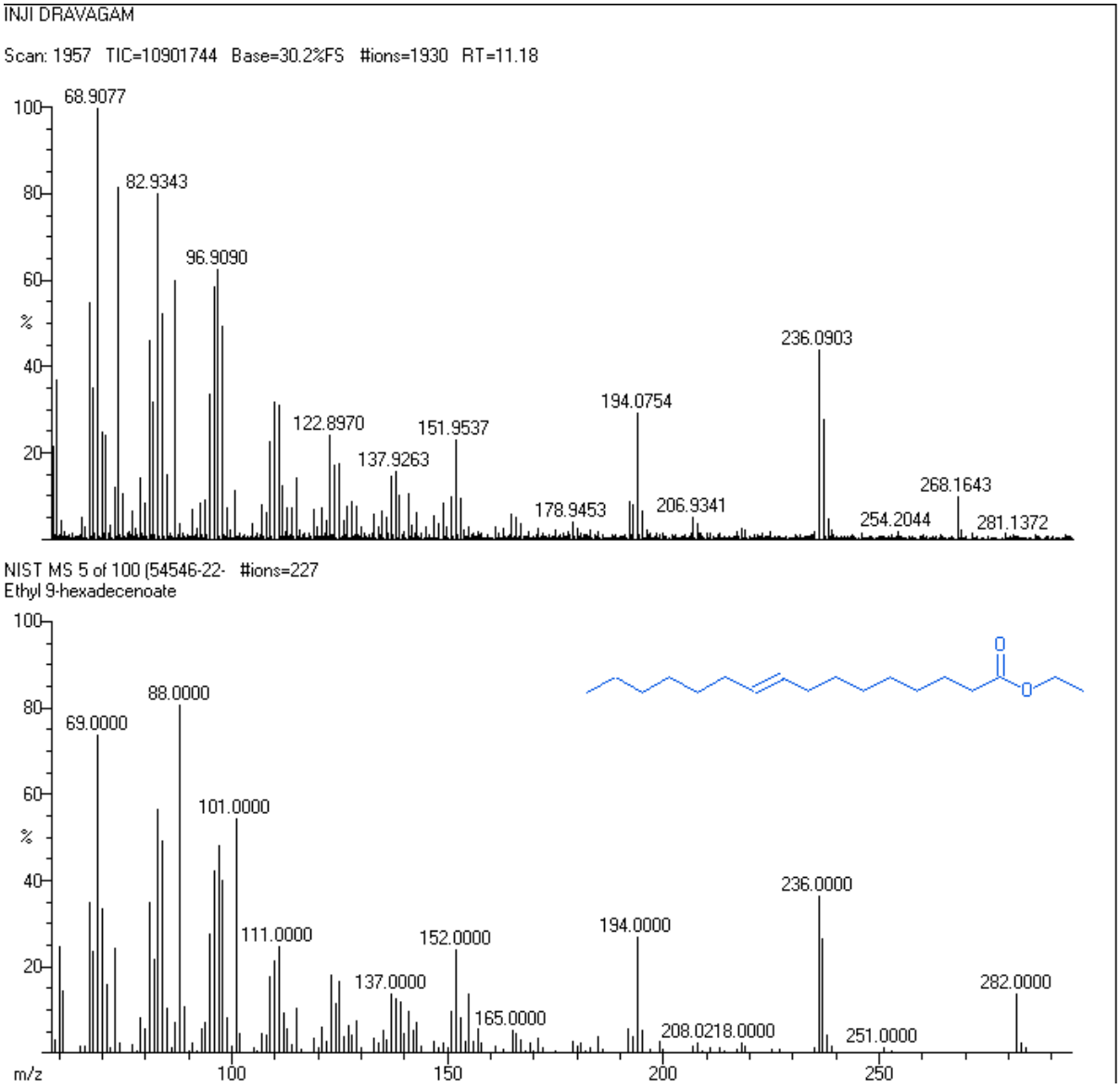
The results Shows the quantitative analysis of the elements present in ***Inji Dravagam* .** The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of ***Inji Dravagam*.** Hence the drug *Inji Dravagam* is considered as a safe drug.

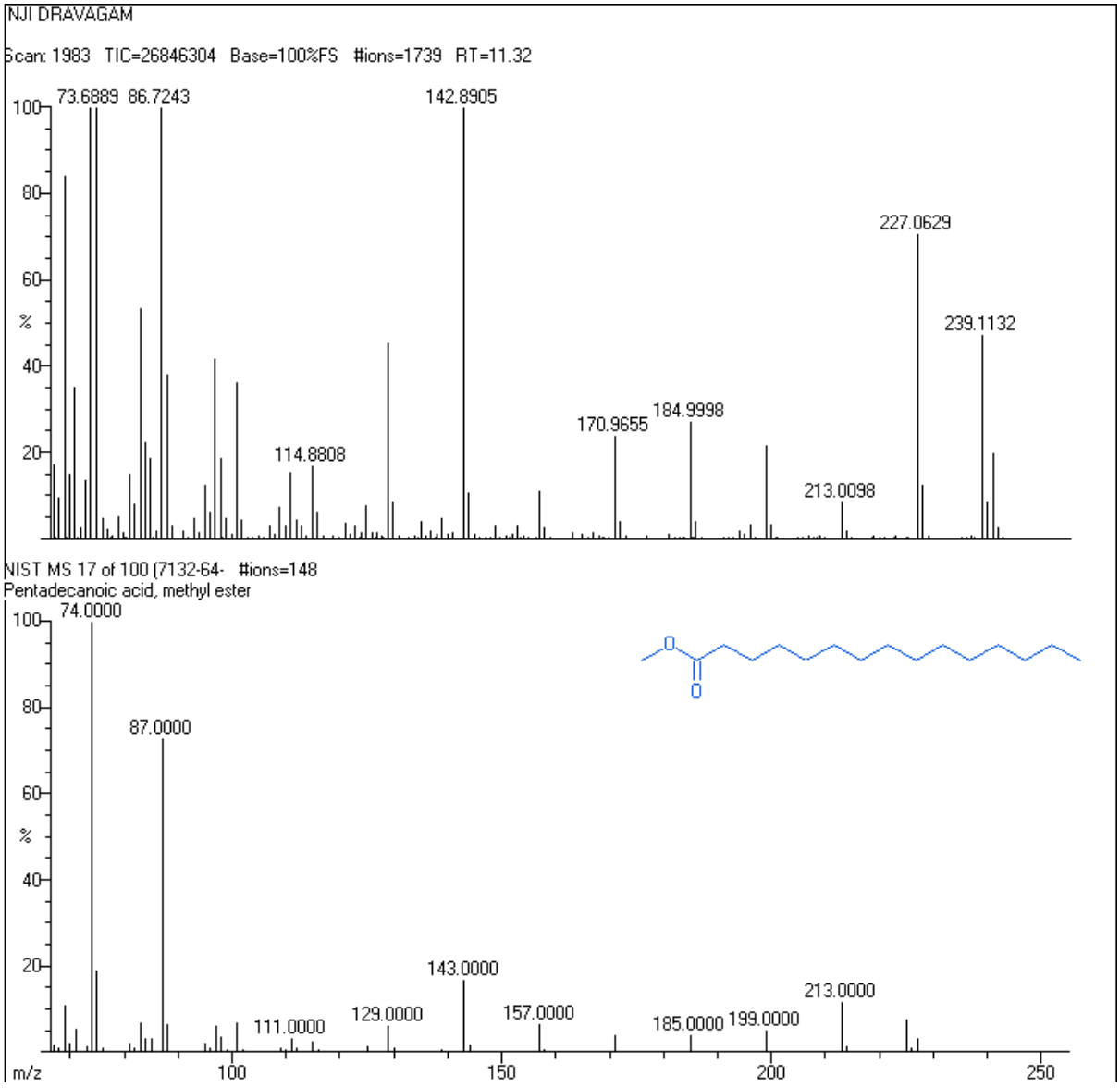
6.6 Gas chromatography of *Inji Dravagam*

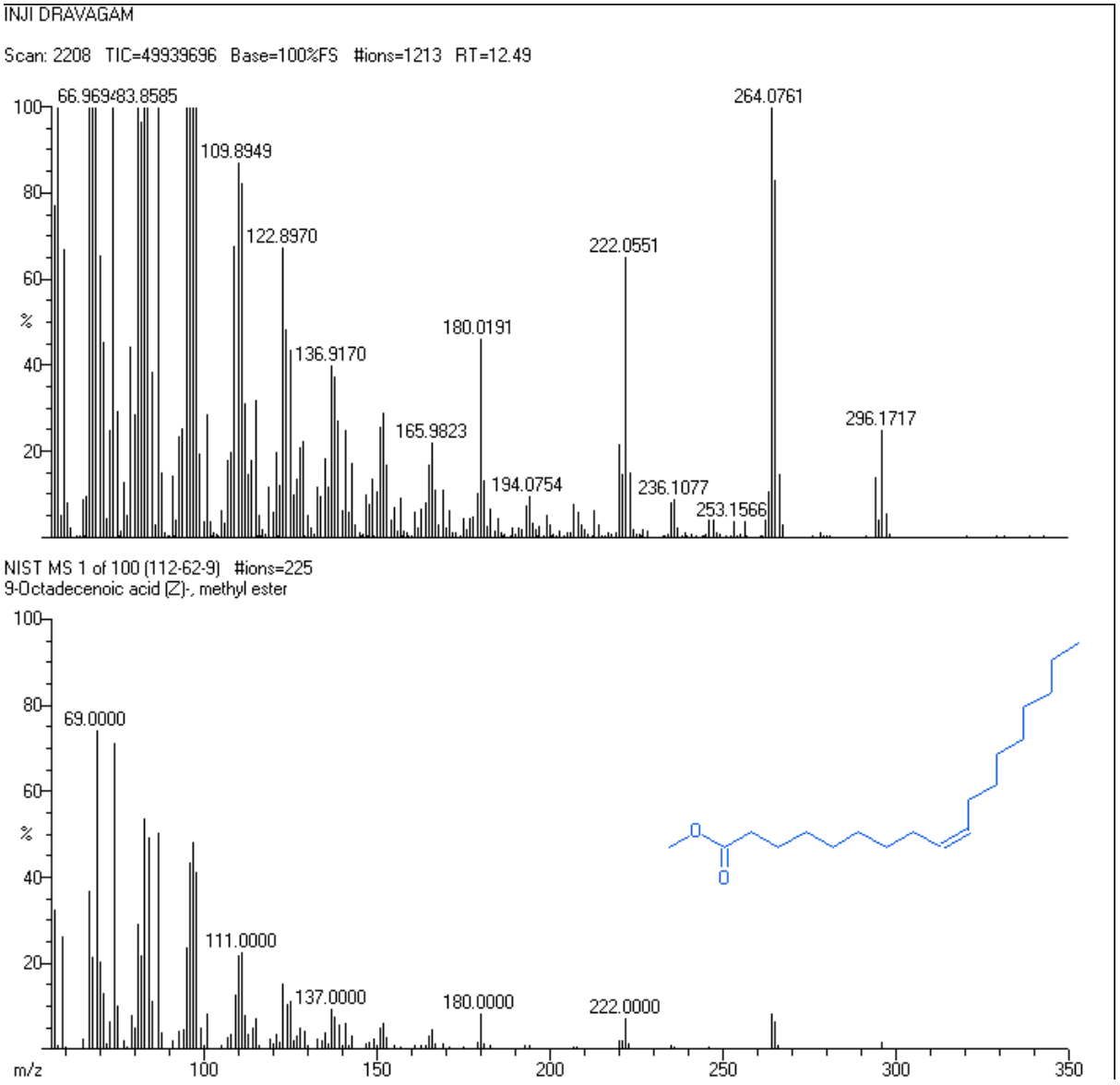


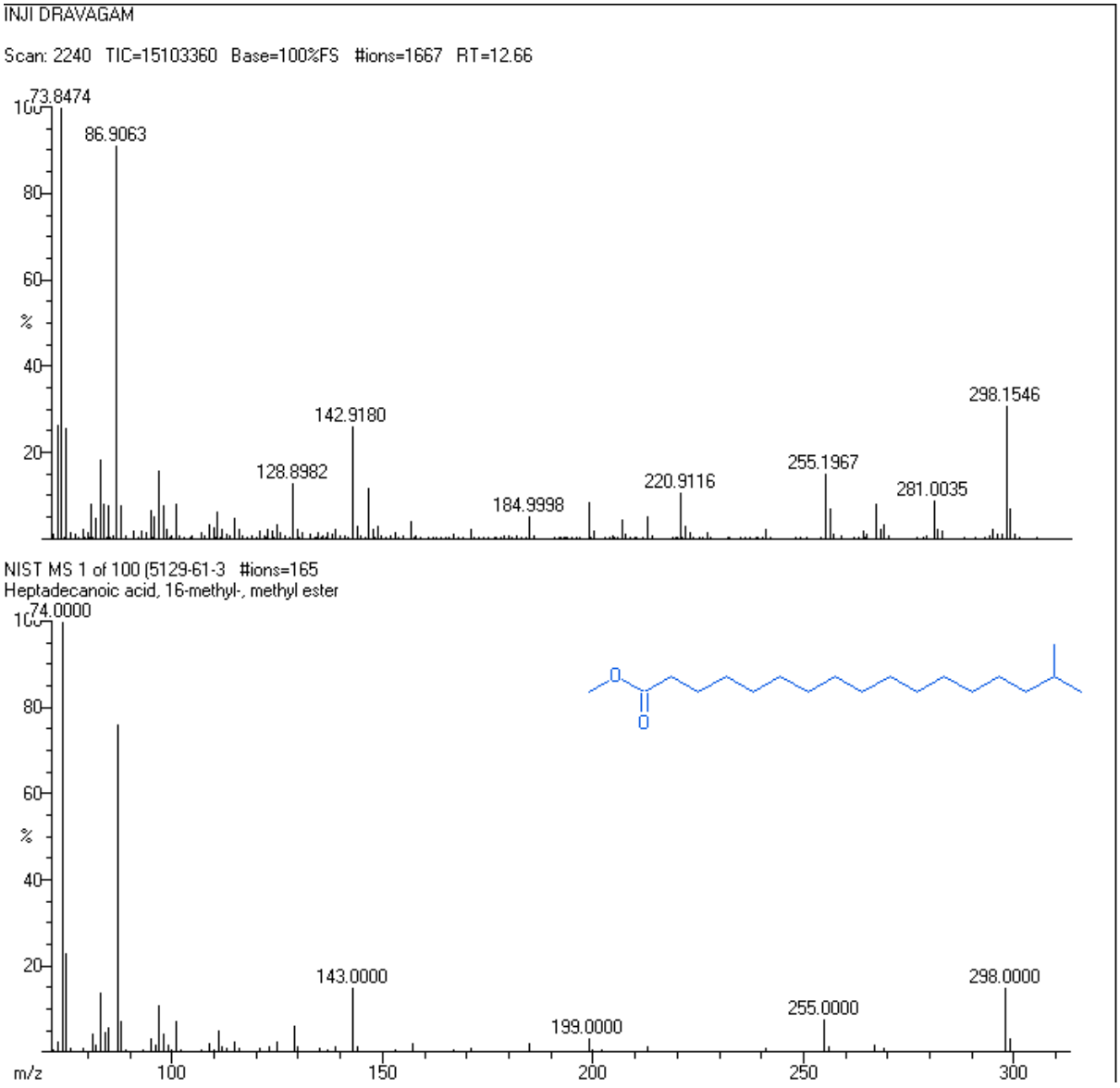

INJI DRAVAGAM
Scan: 1574 TIC=8273152 Base=65.5%FS #ions=2091 RT=9.19
216.9777
232.0748
57.7008
134.8829
NIST MS 1 of 100 (55162-49- #ions=107
Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)methyl]-
232.0000
189.0000
81.0000
107.0000
%
m/z
50
100
150
200
250


NJI DRAVAGAM
Scan: 1799 TIC=8072400 Base=22.5%FS #ions=2152 RT=10.36
82.9448
57.7048
96.9259
124.9285
138.9325
167.98
178.9682
218.9797
234.1019
252.2545
NIST MS 1 of 100 (56554-88- #ions=226
5-Octadecenal
97.0000
147.0000
221.0000
252.0000
%
m/z


INJI DRAVAGAM
Scan: 1957 TIC=10901744 Base=30.2%FS #ions=1930 RT=11.18
68.9077
82.9343
96.9090
122.8970
137.9263
151.9537
178.9453
194.0754
206.9341
236.0903
254.2044
268.1643
281.1372
NIST MS 5 of 100 (54546-22- #ions=227
Ethyl 9-hexadecenoate
69.0000
88.0000
101.0000
111.0000
137.0000
152.0000
165.0000
194.0000
208.0218.0000
236.0000
251.0000
282.0000
%
m/z


NJI DRAVAGAM
Scan: 1983 TIC=26846304 Base=100%FS #ions=1739 RT=11.32
73.6889
86.7243
142.8905
227.0629
239.1132
170.9655
184.9998
114.8808
213.0098
NIST MS 17 of 100 (7132-64- #ions=148
Pentadecanoic acid, methyl ester
74.0000
87.0000
143.0000
213.0000
111.0000
129.0000
157.0000
185.0000
199.0000
%
m/z
100
150
200
250


INJI DRAVAGAM
Scan: 2208 TIC=49939696 Base=100%FS #ions=1213 RT=12.49
66.9694
83.8585
109.8949
122.8970
136.9170
165.9823
180.0191
194.0754
222.0551
236.1077
253.1566
264.0761
296.1717
%
NIST MS 1 of 100 (112-62-9) #ions=225
9-Octadecenoic acid (Z)-, methyl ester
69.0000
111.0000
137.0000
180.0000
222.0000
O
O
m/z
100
150
200
250
300
350


INJI DRAVAGAM
Scan: 2240 TIC=15103360 Base=100%FS #ions=1667 RT=12.66
73.8474
86.9063
142.9180
128.8982
184.9998
220.9116
255.1967
281.0035
298.1546
NIST MS 1 of 100 (5129-61-3 #ions=165
Heptadecanoic acid, 16-methyl-, methyl ester
74.0000
143.0000
199.0000
255.0000
298.0000
%
m/z
100
150
200
250
300

Interpretation:

Through GC-MS analysis can found the name molecular weight and structure of the test drug *Inji Dravagam.*

This image (table: 7) shows the characteristic gas chromatogram of *Inji Dravagam*. In this sample, there are 6 compound were identified, they are the following,

(1) Furan,2-(2-ethoxy-3,4-dimethyl-2-cyclohexo-1-cylidene)methyl)

(2) 5 Otadecenal

(3) Ethyl 9-hexadecenoate

(4) Pentadecandoic acid, methyl ester

(5) 9-octadecenoic acid(Z),methyl ester

(6) Heptadecanoic acid, 16, methyl, methyl ester

Table: 7 Compounds identified in the trial drug *Inji Dravagam*

S.NO	Rt	Name of the compound	Molecular formula	Molecular weight
1	9.19	Furan,2(2-ethoxy-3,4-dimethyl-2-cyclohexo-1-cylidene	$C_{15}H_{20}O_2$	232.0748
2	10.36	5 Otadecenal	$C_{18}H_{34}O$	252.2545
3	11.18	Ethyl 9-hexadecenate	$C_{18}H_{34}O_2$	281.1372
4	11.32	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	239.1132
5	12.49	9-Octadecenoic[Z] methyl ester]	$C_{19}H_{36}O_2$	296.1717
6	12.66	Heptadecanoic acid, 16-methyl- methyl ester	$C_{19}H_{38}O_2$	298.1546

TOXICOLOGY STUDY ON *INJI DRAVAGAM*

7.1 Acute oral toxicity study of *Inji dravagam*

Table 8: Dose finding experiment and its behavioural Signs of Toxicity in wistar albino rats

No	Dose ml/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	10	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2.Aggressiveness 3.Pilo erection 4.Grooming 5.Gripping 6.Touch Response 7.Decreased Motor activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18. Writhing 19.Respiration 20.Mortality

+ Presence of Activity

-Absence of Activity

All the data were summarized in the form of table (7) revealed no abnormal signs and behavioral changes in rats at the dose of 1, 5, 10, ml/kg body weight administered orally.

Table: 9 Body weight (g) of albino rats exposed to *Inji Dravagam* for 28 days

Dose (mg/kg/day)		Days			
	1	7	14	21	28
Control	111.20±3.27	112.33±1.51	112.33±2.34	112.66±1.51	114.00±1.41
Mid dose	112.33±1.51	113.16±0.98	115.08±0.86	115.16±0.75	116.66±0.82
High dose	112.66±2.07	113.33±2.07	114.50±1.38	115.50±0.84	116.82±1.02

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10

Table: 10 Water (ml/day) intake of albino rats exposed to *Inji Dravagam* for 28 days

Dose(mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	35.12±0.75	35.5±0.55	36.0±0.63	37.0±0.89	35.5±2.17
Mid dose	38.0±1.41	40.16±1.28	40.5±0.84	45.0±1.10	44.66±3.27
High dose	41.0±1.10	42.66±1.03	44.33±1.51	44.0±2.83	46.0±2.83

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10

Table: 11 Food (g/day) intake of albino rats exposed to *Inji Dravagam* for 28 days

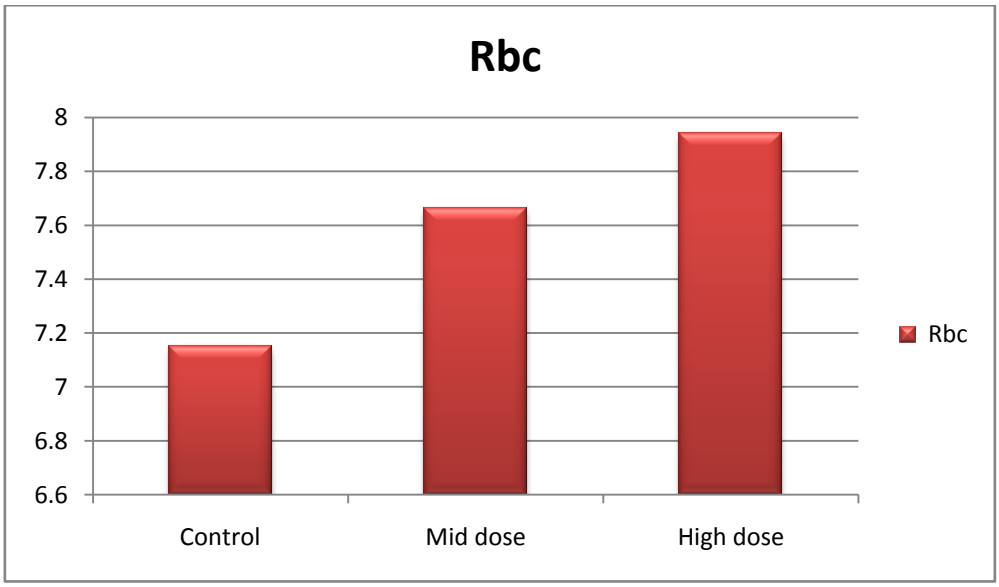
Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	37.46±0.78	36.48±1.63	41.35±0.91	41.28±0.99	42.65±2.92
Mid dose	40.23±0.23	42.2±0.24	42.86±0.90	42.91±1.69	43.73±1.16
High dose	41.38±0.58	41.96±0.40	43.11±0.97	43.53±1.02	44.7±1.60

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=10

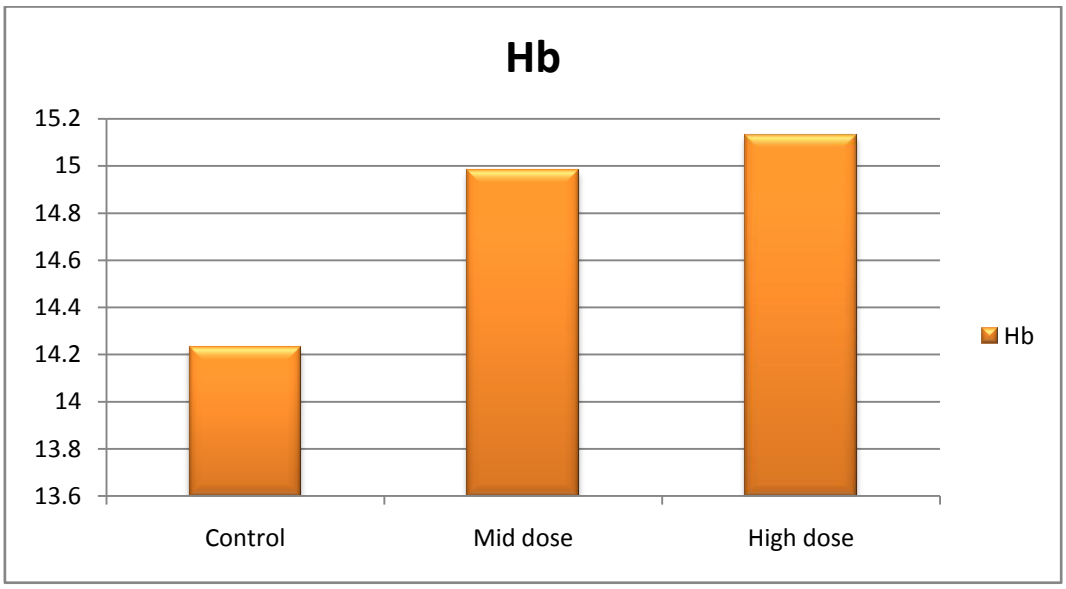
Table: 12Hematological parameters after 28days treatment with *Inji Dravagam* in rats

Parameters	Control	Mid dose	High dose
Red blood cell(mm^3)	7.15±0.15	7.66±0.28	7.94±0.38
HB(%)	14.23±0.21	14.98±0.28	15.13±0.21
Leukocyte ($x10^6$/ml)	10146±51.49	10179±22.10	10269±95.32
Platelets/UL	1418±8.40	1281±33.10	1271±37.08
MCV(gl)	51.57±0.98	54.42±0.63	54.45±2.19
PCV	35.02±0.04	35.62±0.12	35.60±0.10

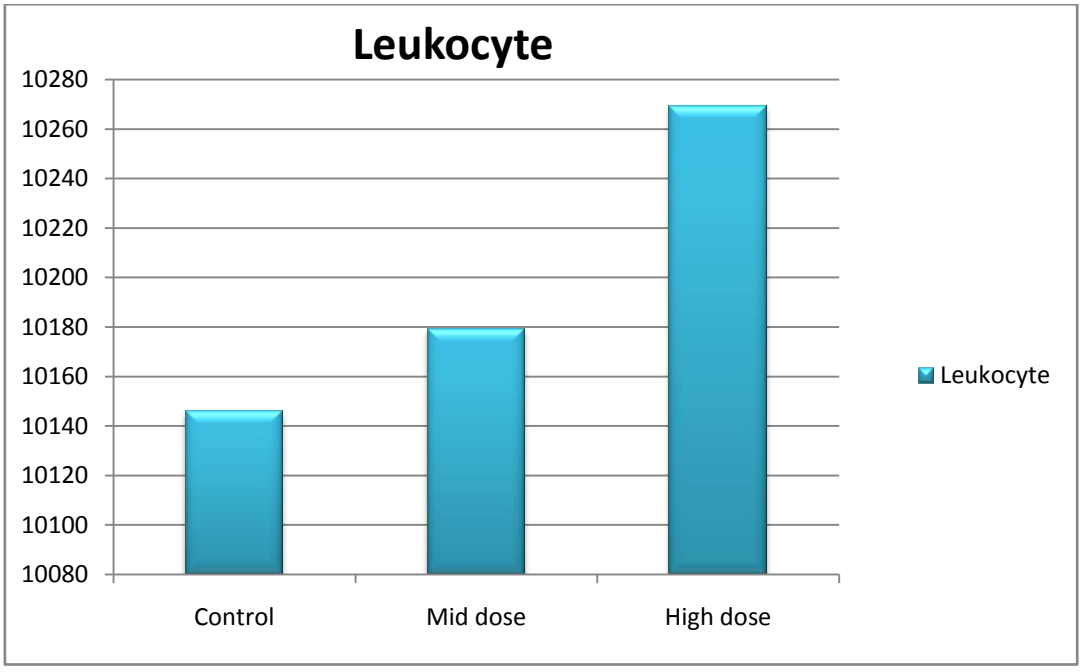
Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05 ;**p<0.01.N=10



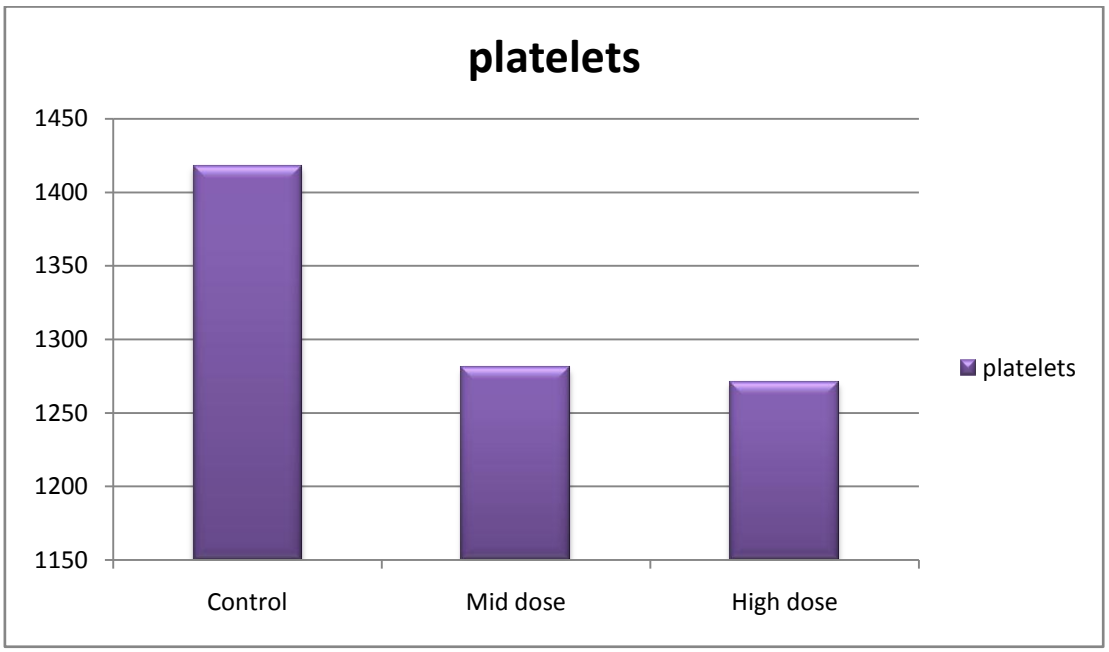
The mean value of T.RBC of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Days Toxic study.



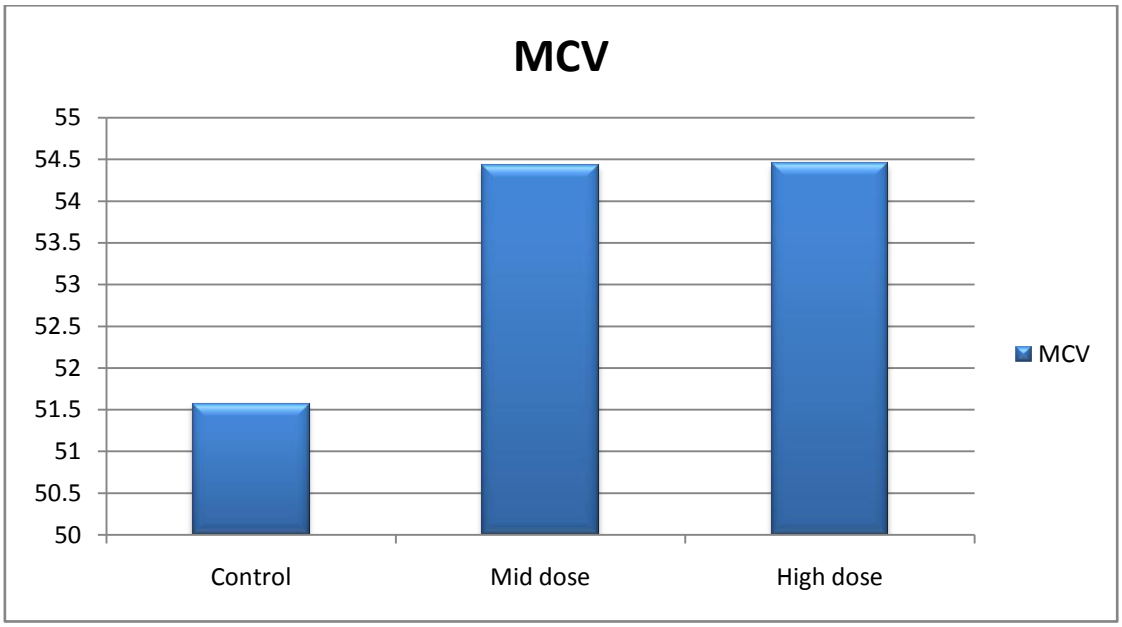
The mean value of HB of control and treated groups of wistar albino rats exposed to *Inji Dravagam in* Repeated Oral 28 Days Toxic study.



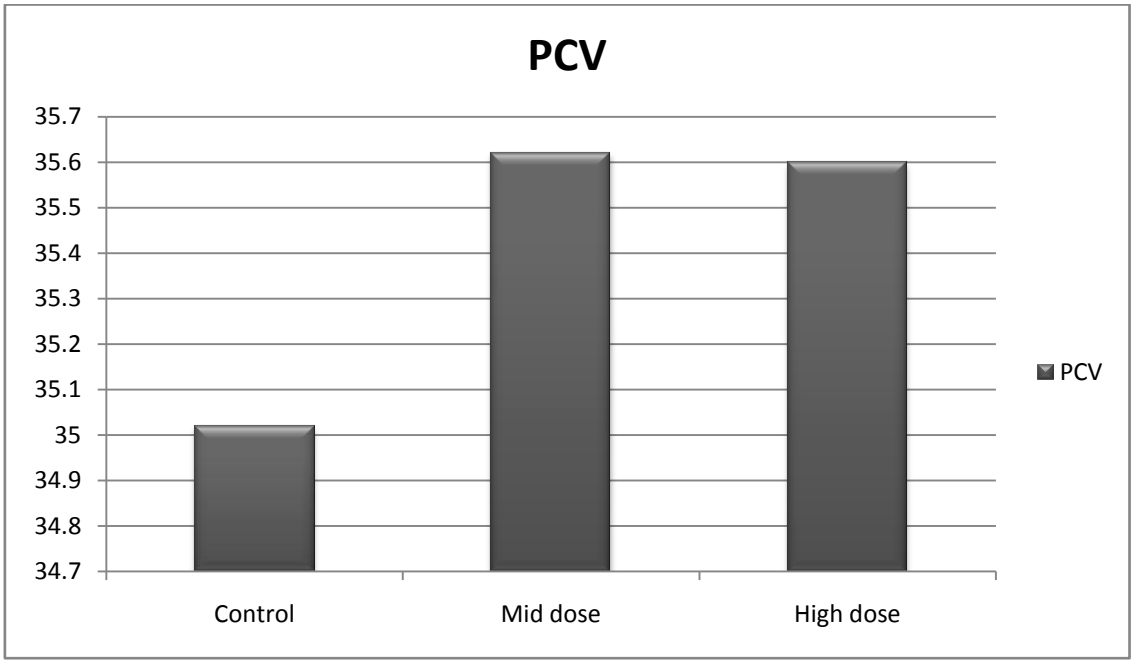
The mean value of Leucocytes of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Days Toxic study.



The mean value of Platelets of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Days Toxic study.



The mean value of MCV of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Days Toxic study.



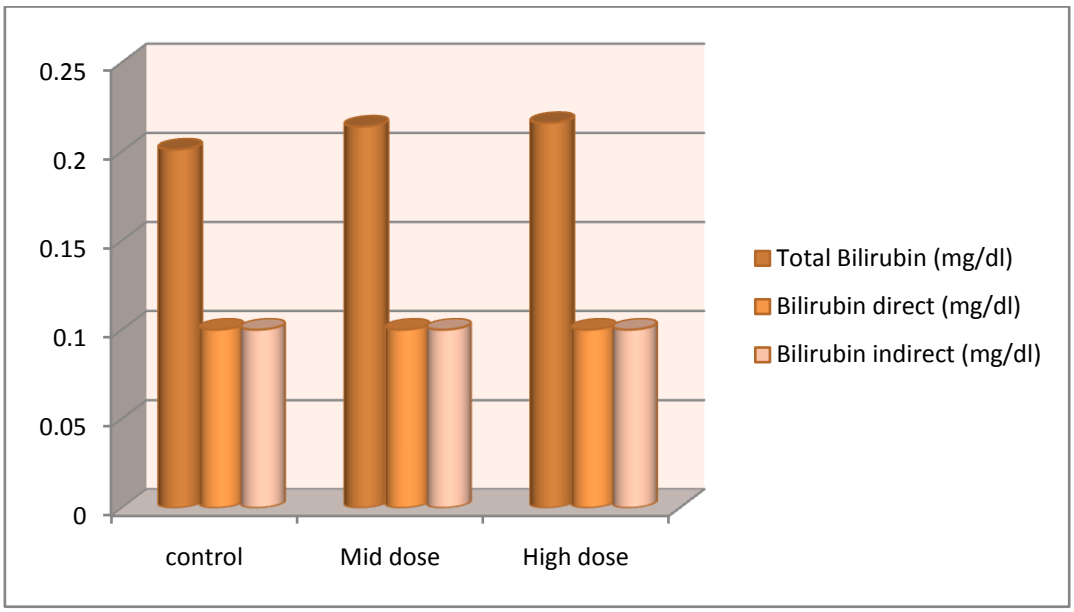
The mean value of PCV of control and treated groups of wistar albino rats exposed to *Inji Dravagam in* Repeated Oral 28 Days Toxic study.

Table 13:Effect of treatment with *Inji Dravagam* on biochemical parameters:

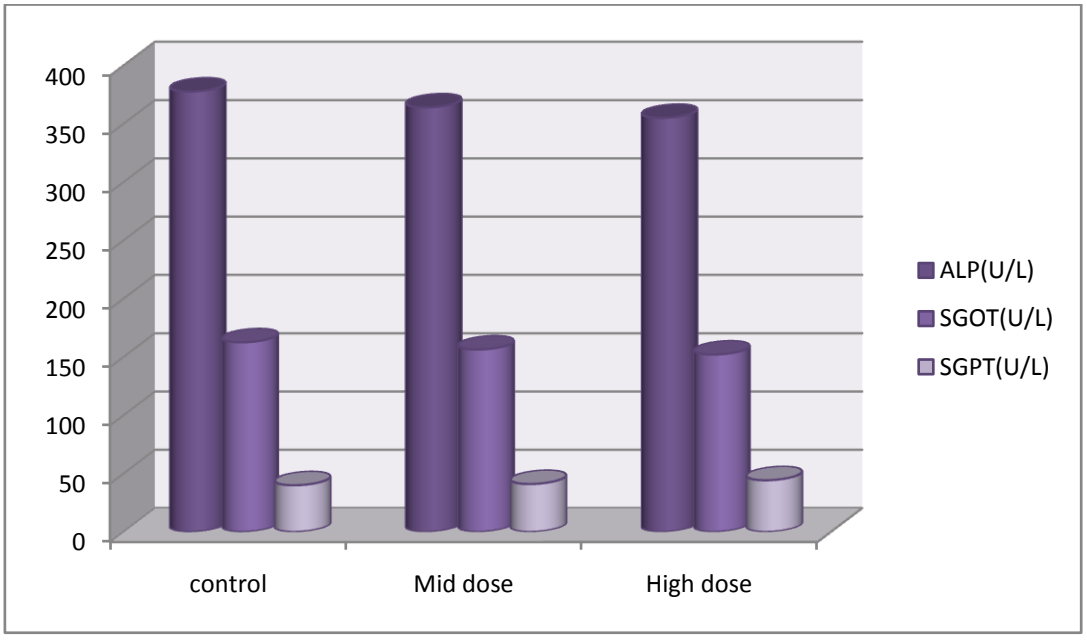
LFT

Dose(mg/kg)	Control	Mid dose	High dose
Total Bilirubin (mg/dl)	0.2011±0.04	0.214±0.01	0.216±0.01
Bilirubin direct(mg/dl)	0.1±0.02	0.1±0.02	0.1±0.04
Bilirubin indirect(mg/dl)	0.1±00	0.1±00	0.1±00
ALP(U/L)	377.6±40.27	364.00±30.35	355.66±4.63
SGOT(U/L)	162.50±2.17	156.33±1.51	152.00±1.79
SGPT(U/L)	39.90±1.01	40.97±0.97	44.20±0.61
Total protein(g/dl)	10.70±0.55	9.36±0.21	9.18±0.04
Albumin(g/dl)	3.06±0.03	3.06±0.03	3.07±0.04
Globulin(g/dl)	6.06±0.05	5.79±0.09	5.27±0.05

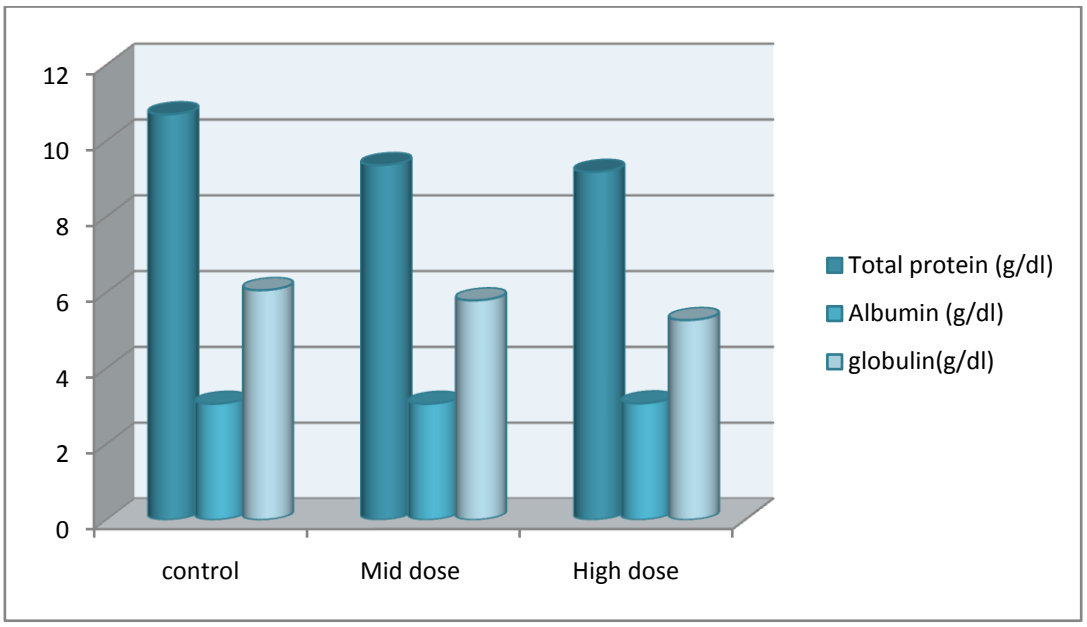
Values are mean of a 10 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=10



The mean value of T. Bilirubin, Bilirubin direct and Bilirubin indirect of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Days Toxic study



The mean value of ALP, SGOT and SGPT of control and treated groups of wistar albino rats exposed to *Inji Dravagam in* Repeated Oral 28 Days Toxic study



The mean value of Total protein, Albumin and Globulin of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 DaysToxic study

Table :14 Effect of *Inji Dravagam* on Renal function test

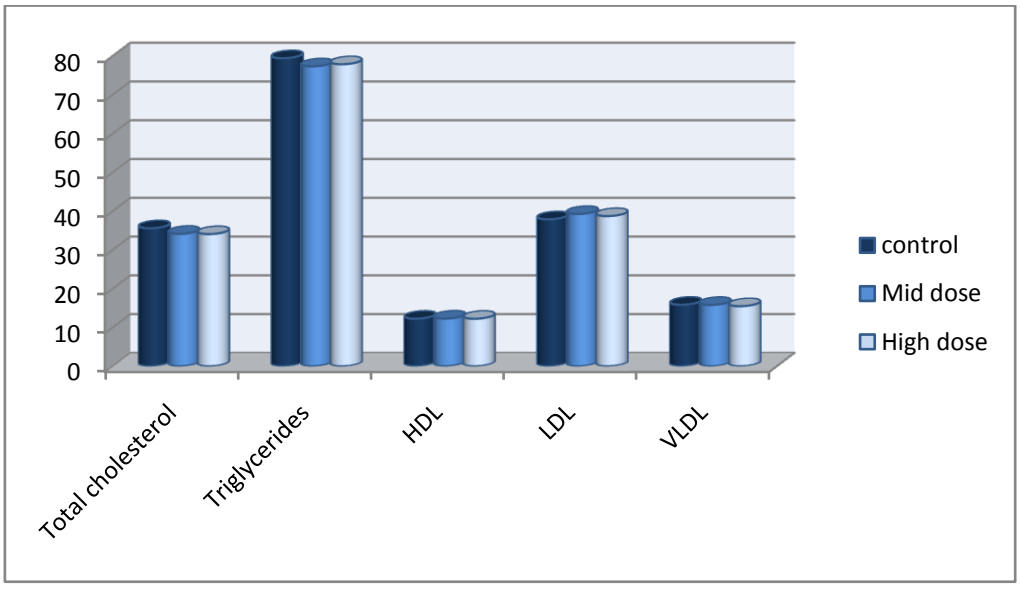
Dose (mg/kg)	**Control**	**Mid dose**	**High dose**
Urea(mg/dl)	53.85±1.39	51.63±0.95	52.36±0.27
Creatinine(mg/dl)	0.72±0.01	0.74±0.02	0.74±0.04
Uric acid(mg/dl)	1.4±0.02	1.4±0.02	1.6±0.04
Na m.mol	130.78±0.72	132.01±0.63	134.53±1.49
K m.mol	18.12±0.07	18.28±0.03	19.23±0.17
Clm.mol	97.57±0.80	99.26±0.23	99.33±1.16

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10

Table 15: Lipid Profile

Parameters	Control	Mid dose	High dose
Total cholesterol (mg/kg)	35.59±0.50	34.14±0.91	34.08±.0.81
HDL(mg/dl)	12.26±0.16	12.28±0.12	12.24±0.12
LDL(mg/dl)	37.90±1.36	39.26±0.89	38.73±1.47
VLDL (mg/dl)	15.85±0.09	15.81±0.11	15.48±1.25
Triglycerides(mg/dl)	79.44±0.01	77.34±2.16	77.89 ±2.29
TC/HDL ratio (g/dl)	2.46±0.12	3.42±1.23	3.20±0.12
Blood glucose (mg/dl)	124.03±0.21	124.17±0.30	124.03±0.11

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10



The mean value of total cholesterol, triglycerides, HDL, LDL, VLDL, of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 28 Day Toxic study

Table 16: Urine Analysis of 28 Days Repeated oral toxicity study of *Inji Dravagam* in wistar albino rats

Parameters	Control	Mid dose	High dose
Transparency	Clear	Slightly turbid	Slightly turbid
Specific gravity	1.010	1.010	1.010
PH	>7.2	>7.2	>7.4
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal
Pus cells	0-cells/HPF	0-cells/HPF	1-cells/HPF
RBC	Nil	Nil	1-cells/HPF
Epithelial cells	Nil	1-cells/HPF	Nil
Crystals	Nil	Nil	Nil
Casts	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen
Colour	yellow	Yellow	Yellow

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=10

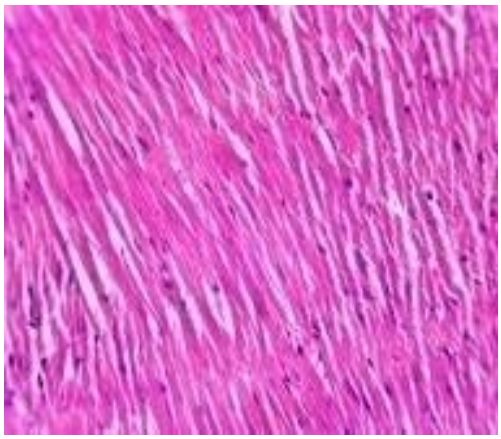
Table 17: Effect of oral administration of a *Inji Dravagam* on organ weight

Dose (mg/kg)	Control	Mid dose	High dose
Liver(g)	4.44±0.12	4.53±0.19	4.58±0.05
Heart (g)	0.39±0.04	0.39±0.03	0.39±0.02
Lung(g)	1.30±0.24	1.30±0.68	1.30±0.88
Spleen (g)	0.48±0.16	0.50±0.18	0.52±0.22
Ovary (g)	1.48±0.04	1.50±0.	1.52±0.04
Testes(g)	1.20±0.13	1.22±0.22	1.22±0.28
Brain(g)	1.36±0.22	1.38±0.24	1.40±0.26
Kidney(g)	0.60±0.04	0.62±0.04	0.64±0.04
Stomach(g)	1.35±0.03	1.38±0.02	1.38±0.02

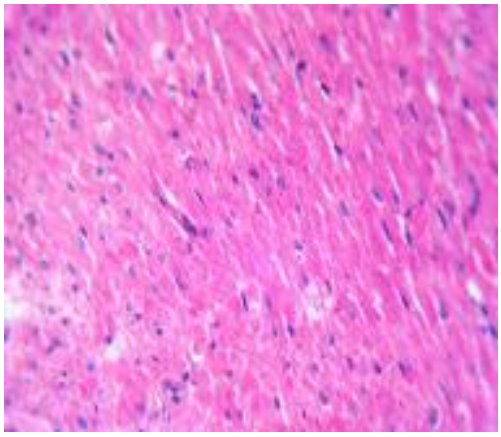
Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10

HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE 28 DAYS REPEATED DOSE ORALTOXICITY STUDY OF INJI DRAVAGAM IN WISTAR ALBINO RATS

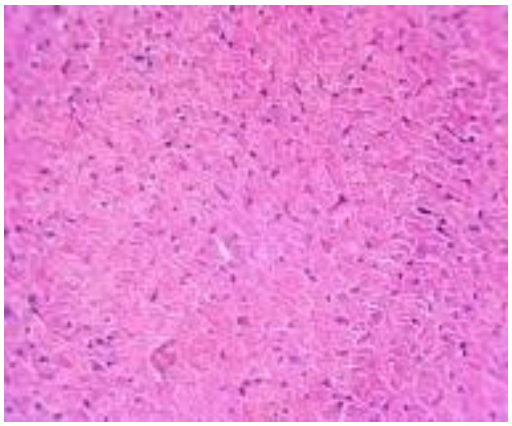
Heart



Control

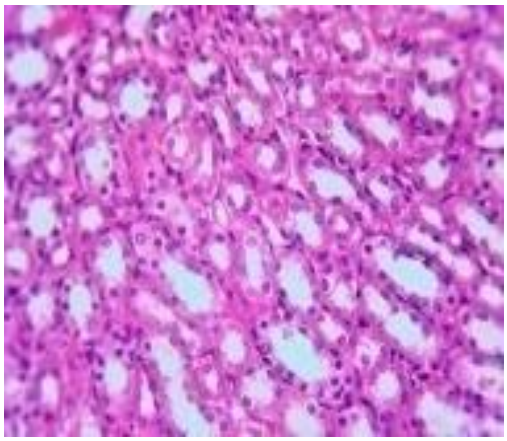


Mild dose

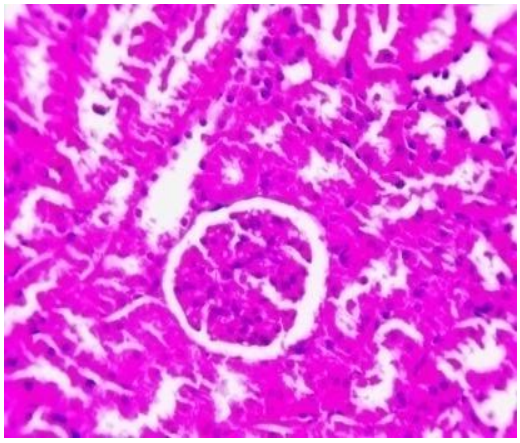


High dose

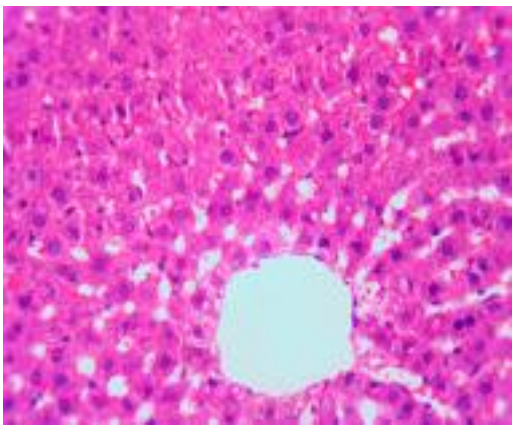
Kidney



Control



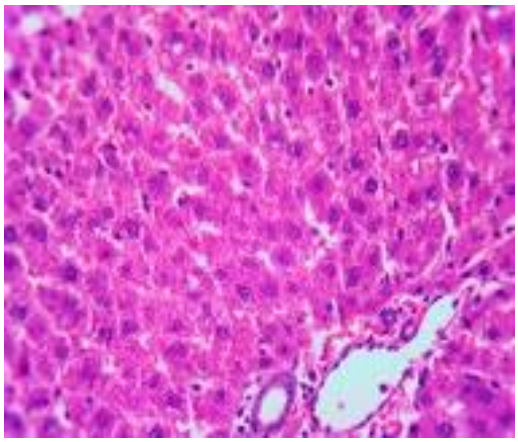
Mild dose



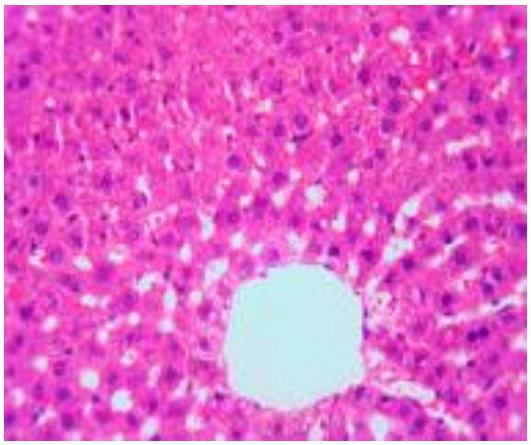
High dose

HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE 28 DAYS REPEATED DOSE ORALTOXICITY STUDY OF INJI DRAVAGAM IN WISTAR ALBINO RATS

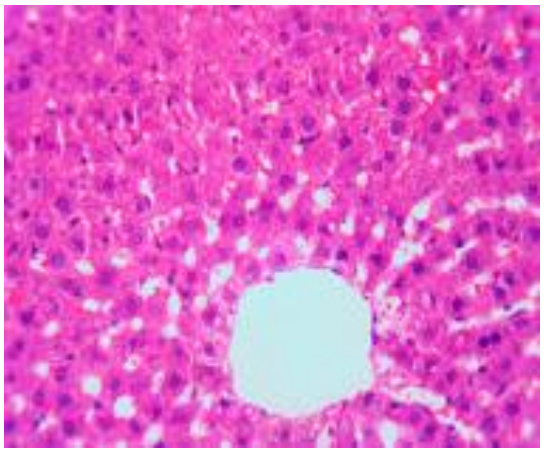
Liver



Control

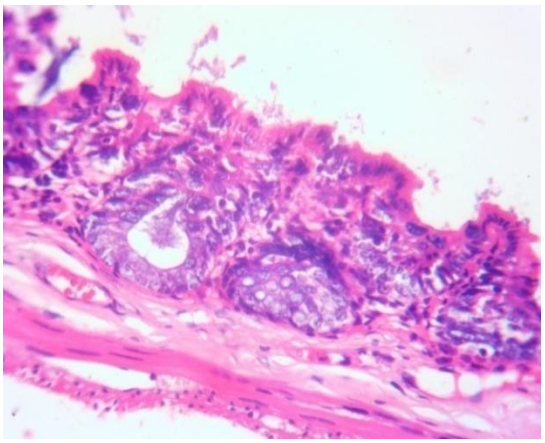


Mild dose

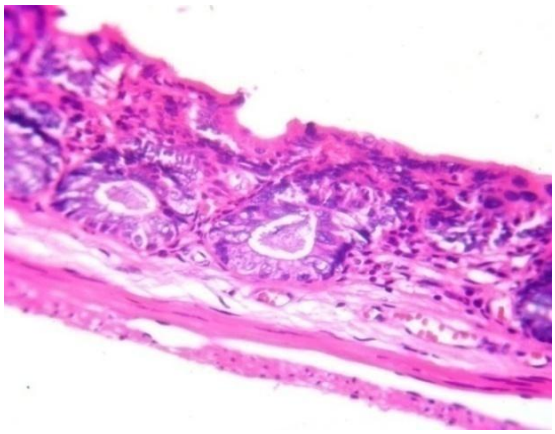


High dose

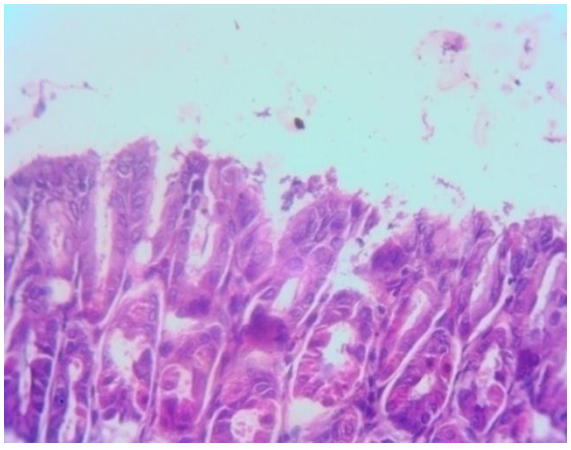
Stomach:



Control



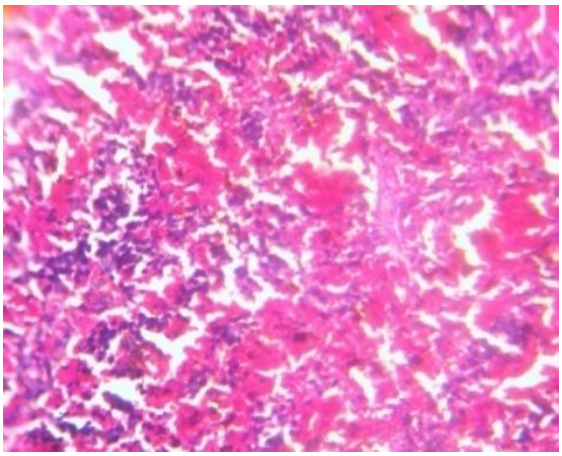
Mild dose



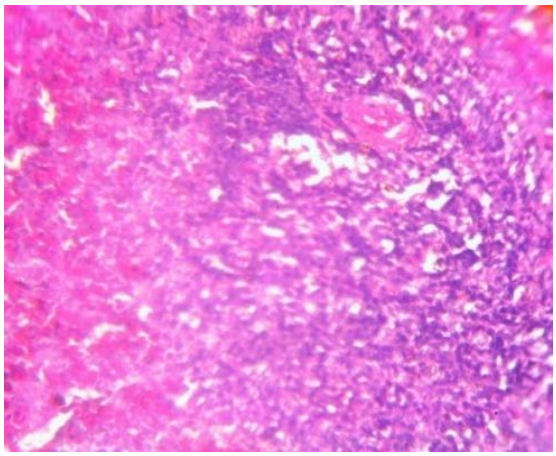
High dose

HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE 28 DAYS REPEATED DOSE ORALTOXICITY STUDY OF INJI DRAVAGAM IN WISTAR ALBINO RATS

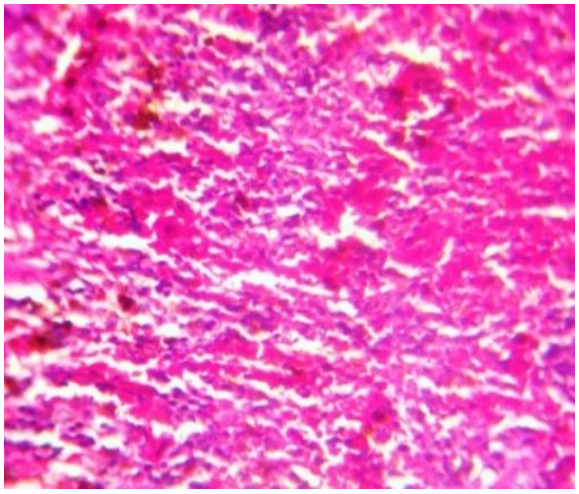
Spleen



Control



Mild dose



High dose

Interpretation:

Heart:

Section of heart from control animal showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei.

In treated group showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei with normal structure.

Kidney:

Section of kidney from control animals showed normal size of glomeruli with normal tubules.

Kidney of treated animals showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.

Liver:

Section of liver from control animals shows No degeneration of hepatocytes, focalsteatosis, and congestion of central vein and inflammation of portal tract.

Liver of treated groups showed No degeneration of hepatocytes, focalsteatosis, no congestion of central vein and inflammation of portal tract

Spleen:

Section of spleen from control animal shows normal granular hemosiderin pigment predominantly within macrophages in the red pulp.

Treated animals showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.

Stomach:

Section of stomach from control animals shows gastric mucosa lined by tall columnar cell with no abnormality.

Stomach of treated group animals shows gastric mucosa lined by tall columnar cell no abnormal changes.

Heart, liver, kidney, and stomach showed no changes in the cellular architecture in treated groups. From the histopathological study no related changes in vital organs are observed in treated groups.

PRECLINICAL TOXICITY RESULTS OF INJI DRAVGAM

Interpretation of Sub-acute toxicity of *Inji dravagam*

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub acute toxicity study. There was no significant change in the body weight for the control and treatment group throughout the dosing period of 28 days.

Intrepratation of hematological investigation

The results of haematological investigations conducted on day 29th day revealed no significant changes in the haematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *Inji dravagam.*

Intrepratation of Biochemical investigation

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits.

Intrepretation of histopathology:

The vital organs such as liver, heart, kidneys, stomach and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

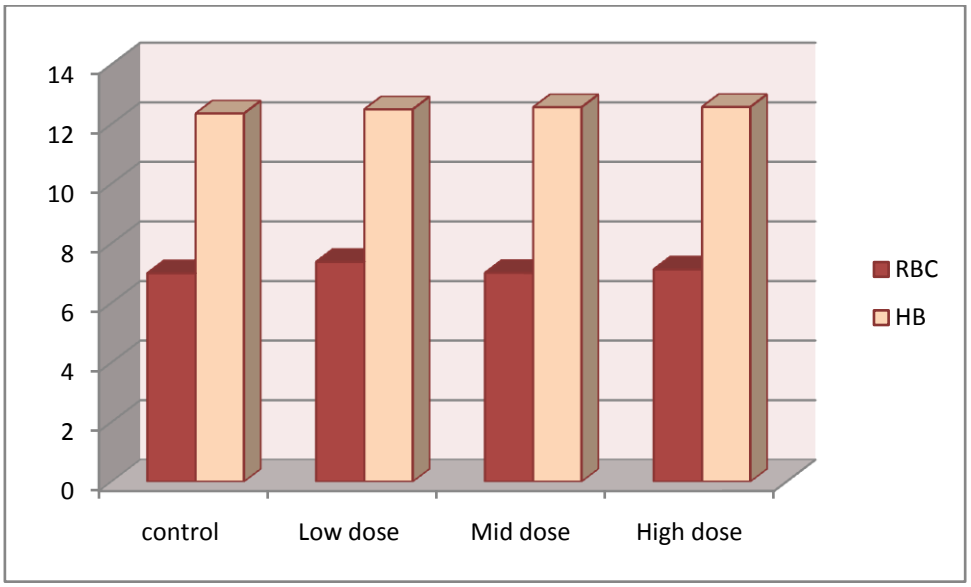
Organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals did not reveal any abnormalities. Histopathology examination did not reveal any abnormal macroscopic changes.

7.3CHRONIC TOXICITY STUDY:

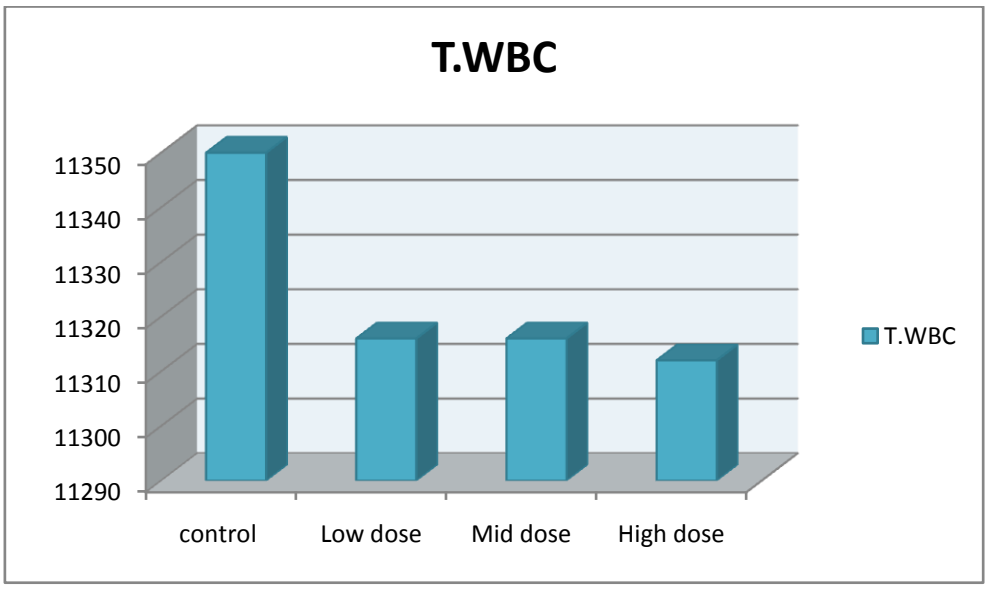
Table:18 Haematological parameters of Wistar albino rats group exposed to *Inji Dravagam* for 90 days

Parameter	Control	1ml/kg	5ml/kg	10ml/kg
RBC (mm3)	7.00±23	7.38±46	7.017±30	7.133±33
PLATELET	3.33±42	3.717±22	3.63±15	3. 78±24
HB(%)	12.52±36	12.50±71	12.57±33	12.58±47
T.WBC($\times 10^6$ /ml)	11350±242.89	11316±462.24	11316±716.70	11312±568.03
PCV	37.57±1.07	37.50±2.13	37.70±96	37.81±1.53
MCV	91.33±2.42	91.33±1.75	91.83±.75	90.33±4.17
MCH	32.17±2.48	32.33±3.07	30.67±1.75	32.50±1.37
MCHC	35.50±1.87	35.50±2.88	36.50±4.76	33.33±2.50

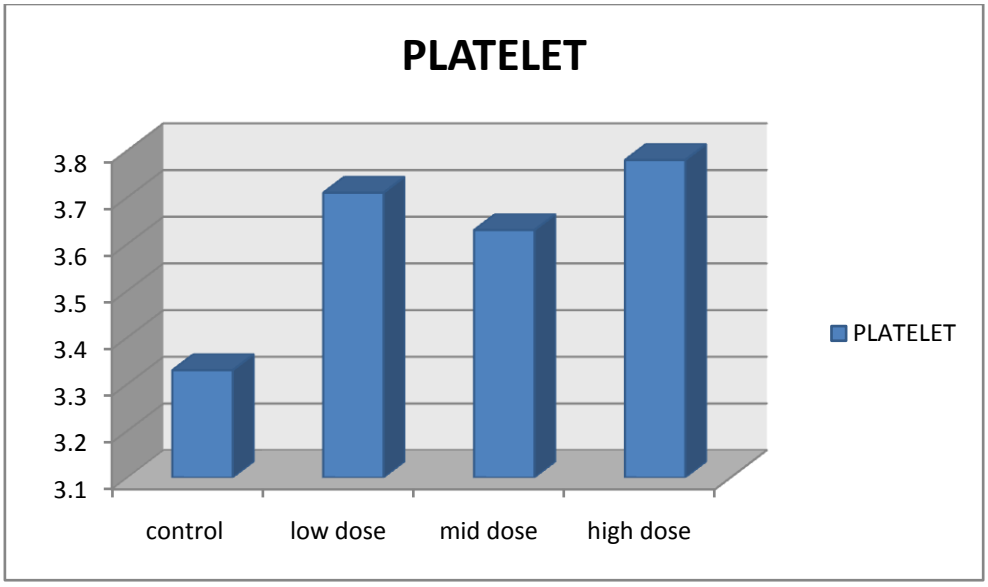
N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)



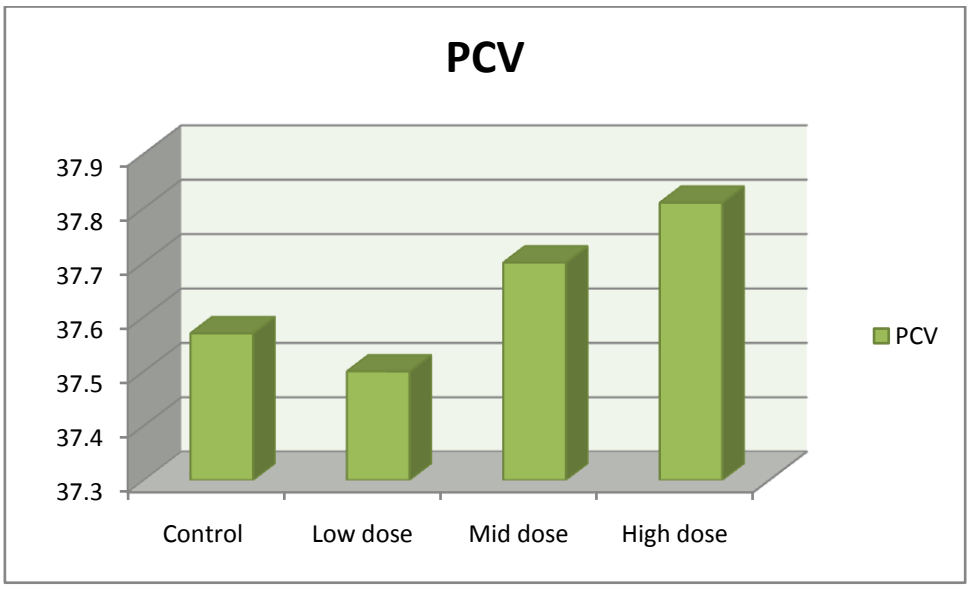
The mean value of RBC and HB control and treated groups of wistar albino rats exposed to Inji Dravagam in Repeated Oral 90 Days Toxicity study.



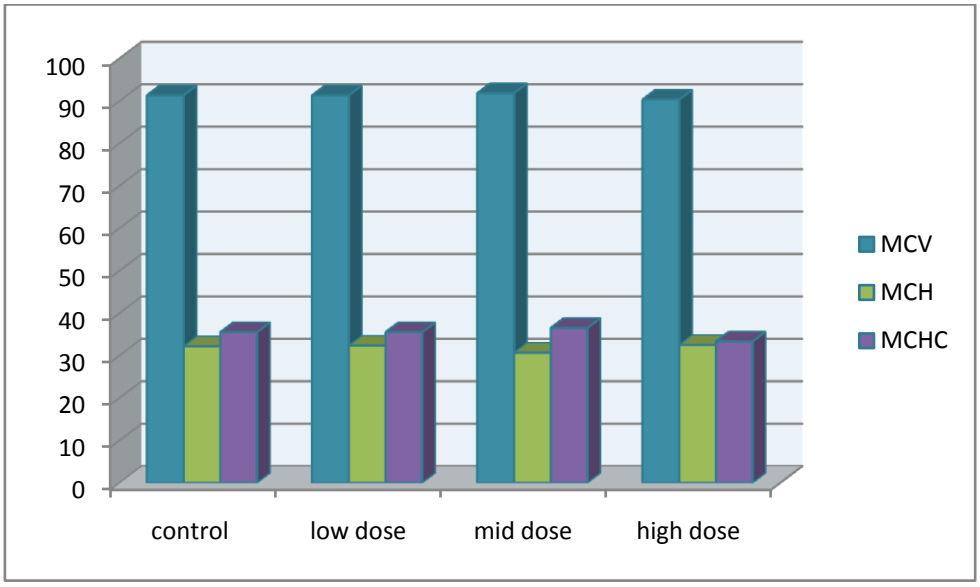
The mean value of T.WBC control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.



The mean value of PLATELET control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.



The mean value of PCV control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.

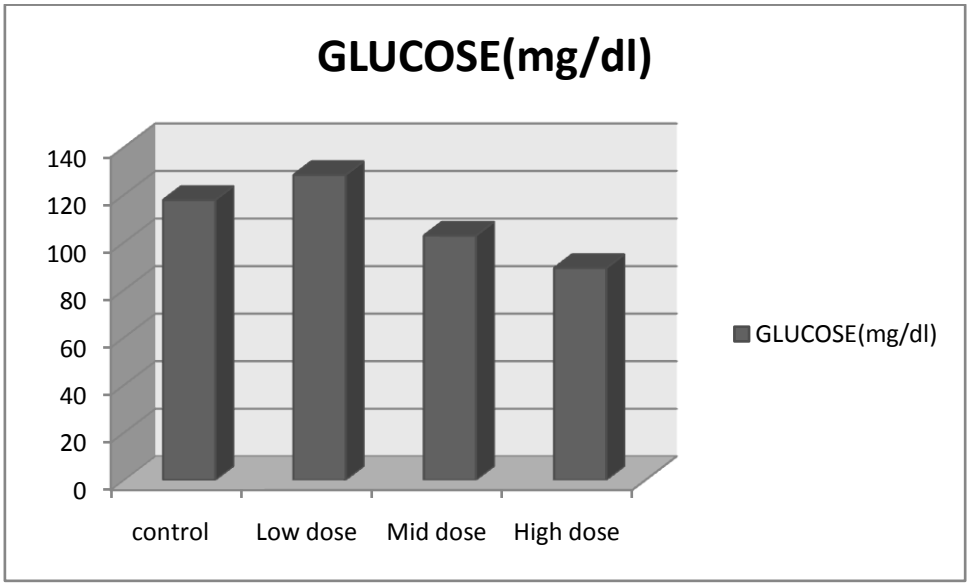


The mean value of MCV, MCH and MCHC control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.

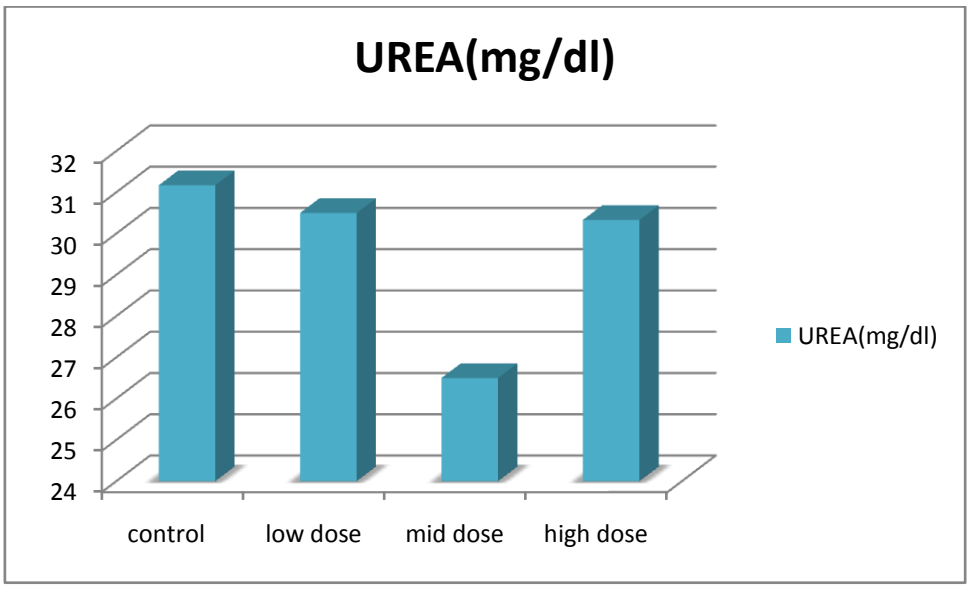
Table: 19 Effect of treatment with *Inji Dravagam* on biochemical parameters

Parameter	Control	1ml/kg	5ml/kg	10ml/kg
GLUCOSE(mg/dl)	117.83±20.29	128.33±13.57	102.83±8.70	89.17±4.62
UREA(mg/dl)	31.17±5.94	30.50±7.817	26.50±6.89	30.33±4.50
CREATININE(mg/dl)	0.55±0.18	0.51±.14	0.51±0.13	0.50±0.16
TOTAL BILIRUBIN(mg/dl)	0.65±0.13	0.85±.0.05	0.81±0.07	0.76±0.15
SGOT U/L	25.50±4.46	25.00±2.68	23.83±6.99	23.50±5.24
SGPT U/L	25.00±4.29	27.67±3.07	27.67±5.57	23.00±7.64
ALP U/L	83.83±9.32	87.50±10.87	90.67±7.03	105.00±5.44
TOTAL CHOLESTEROL(mg/dl)	107.83±9.32	114.17±7.38	120.33±4.88	112.50±11.53
TGL (mg/dl)	138.67±6.37	135.50±6.65	139.00±9.83	134.00±6.81
HDL (mg/dl)	38.38±2.16	46.17±3.76	41.17±2.04	41.33±6.05
LDL (mg/dl)	41.83±9.66	40.83±8.93	51.33±5.78	44.17±16.01
VLDL(mg/dl)	27.73±1.27	27.10±1.33	27.80±1.96	26.63±1.60

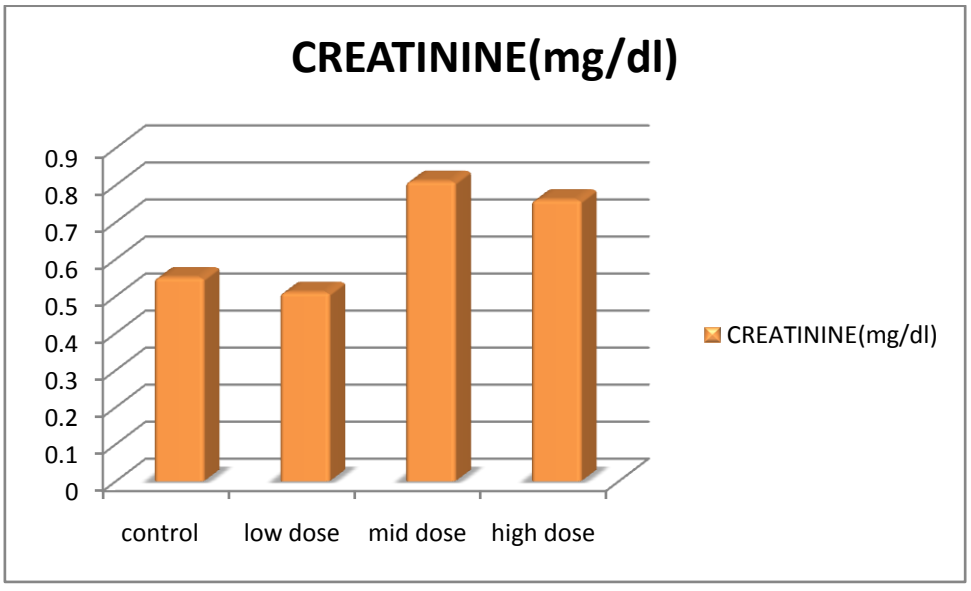
NS- Not Significant, ** ($p > 0.01$), * ($p > 0.05$), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)



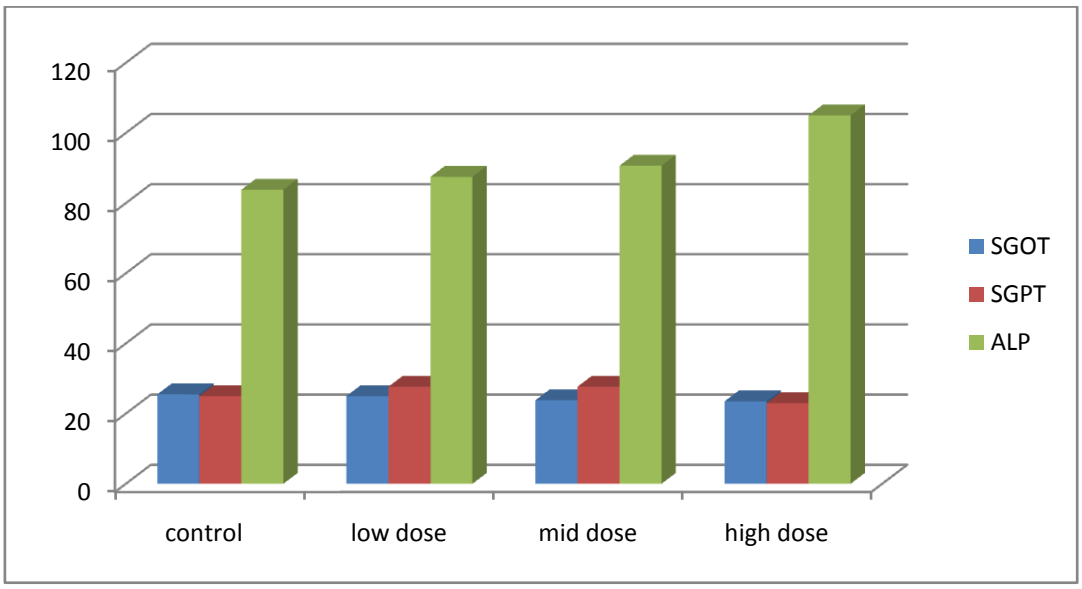
The mean value of GLUCOSE control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.



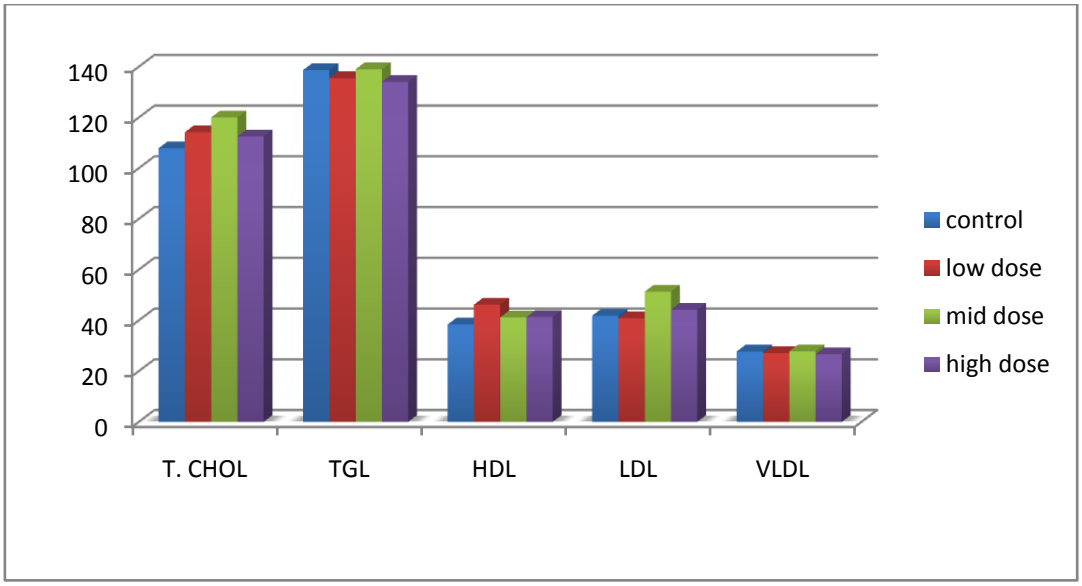
The mean value of UREA control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.



The mean value of CREATININE control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.



The mean value of SGOT, SGPT, ALP, of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.

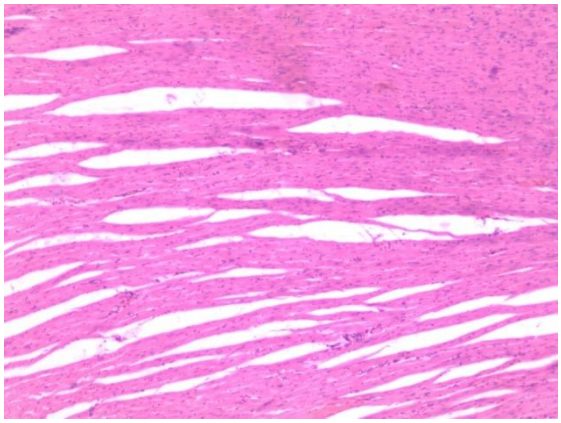


The mean value of TOTAL CHOLESTEROL, TGL, HDL, LDL, VLDL, of control and treated groups of wistar albino rats exposed to *Inji Dravagam* in Repeated Oral 90 Days Toxicity study.

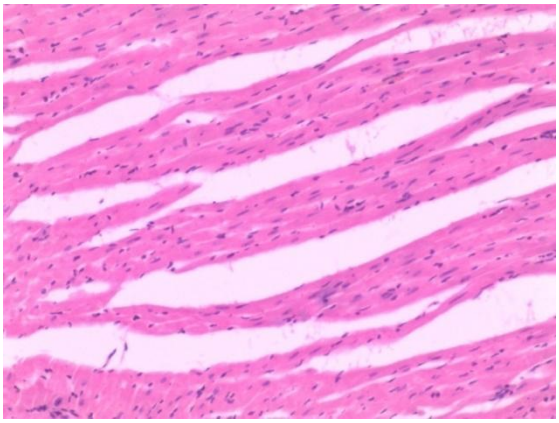
HISTOPATHOLOGICAL OF CONTROL GROUP ANIMALS

Heart

Low Power Magnification 10X

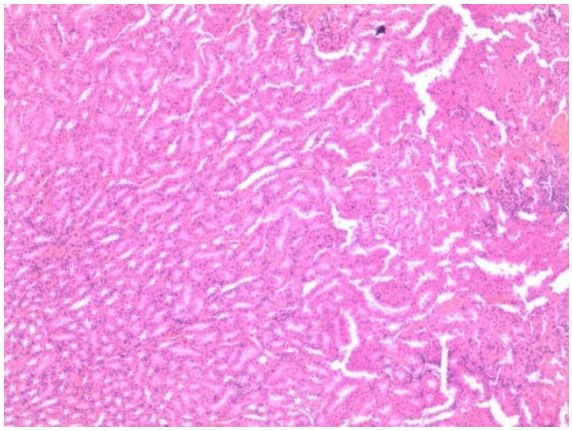


High Power Magnification 40X

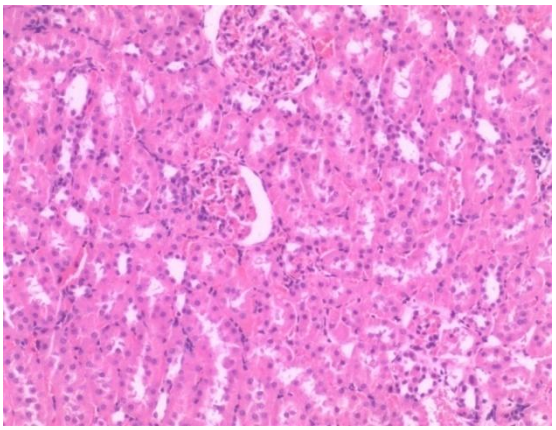


Kidney

Low Power Magnification 10X

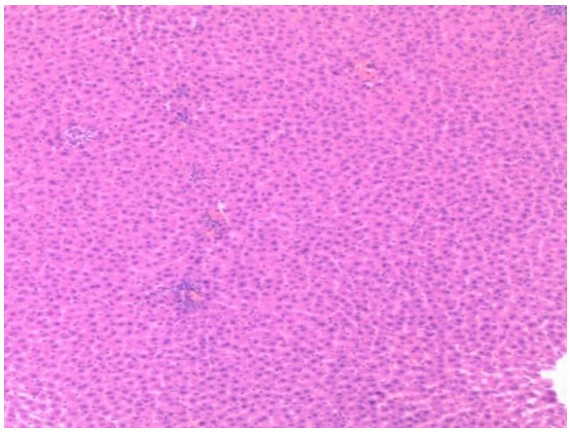


High Power Magnification 40X

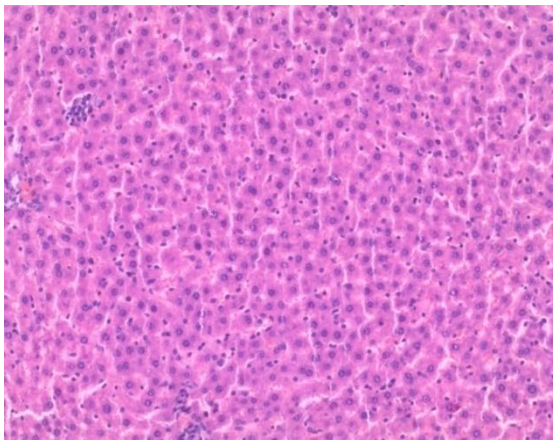


Liver

Low Power Magnification 10X



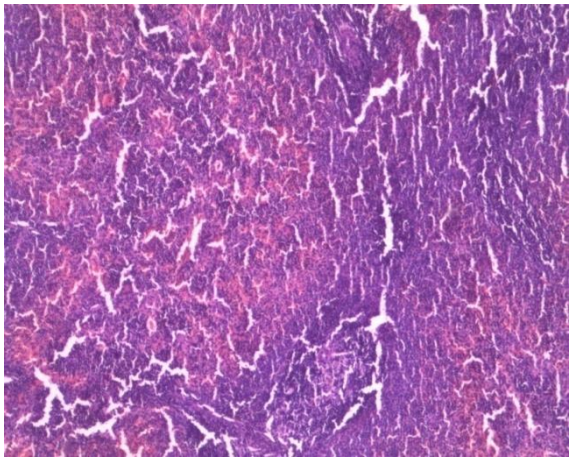
High Power Magnification 40X



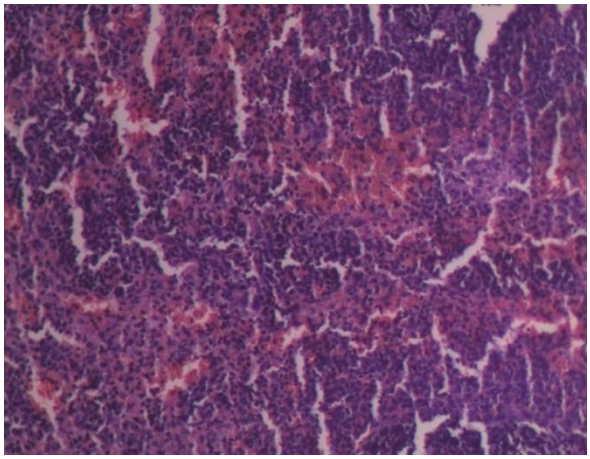
HISTOPATHOLOGICAL OF CONTROL GROUP ANIMALS

Spleen

Low Power Magnification 10X

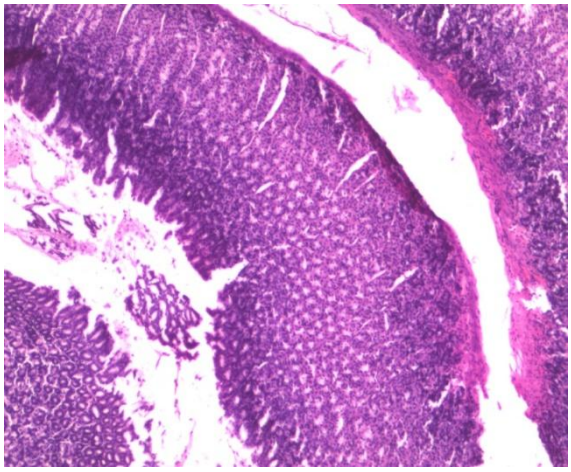


High Power Magnification 40X

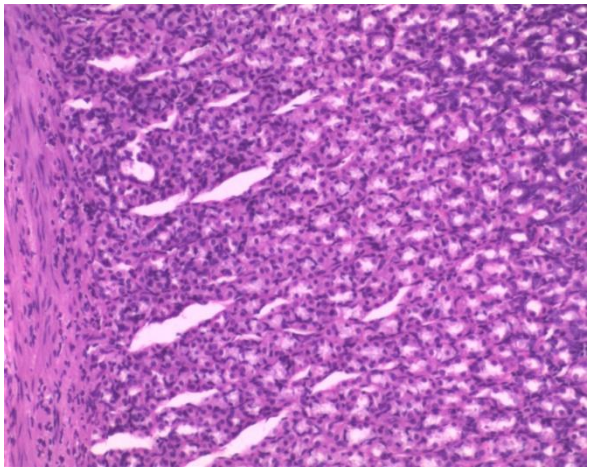


Stomach

Low Power Magnification 10X



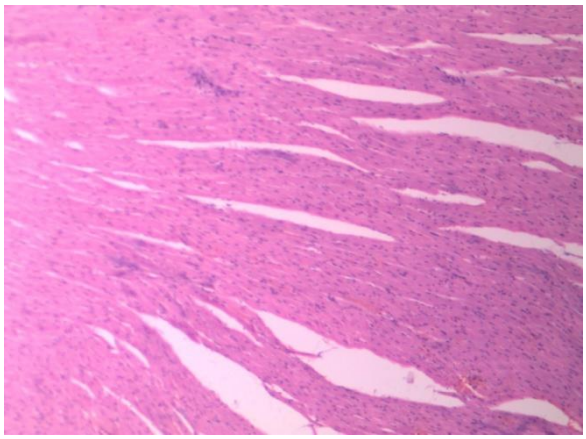
High Power Magnification 40X



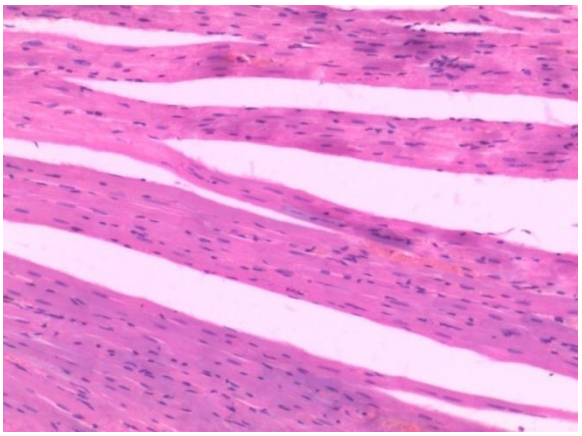
HISTOPATHOLOGY OF HIGH DOSE GROUP ANIMAL

Heart

Low Power Magnification 10X

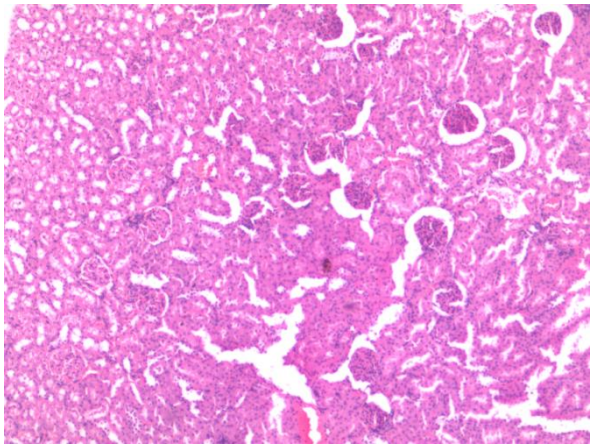


High Power Magnification 40X

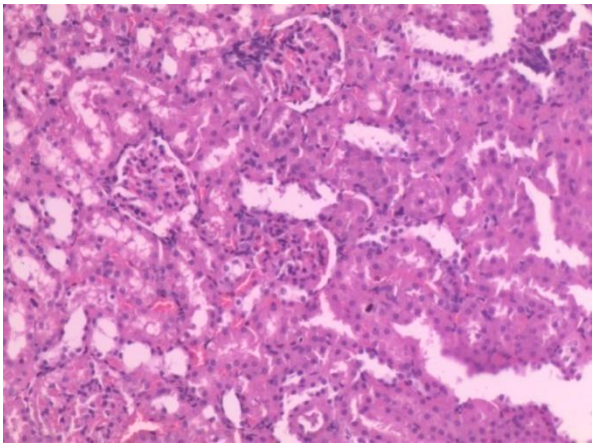


Kidney

Low Power Magnification 10X

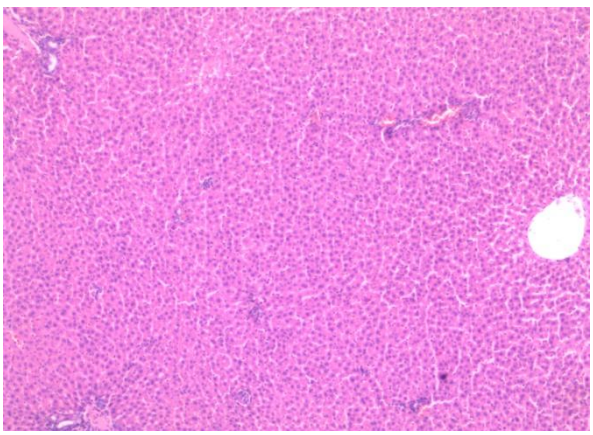


High Power Magnification 40X

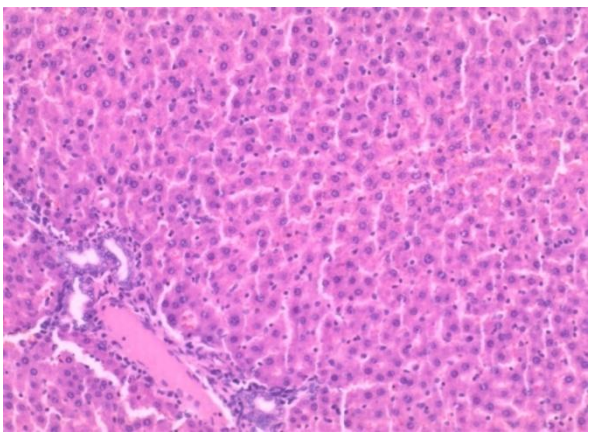


Liver

Low Power Magnification 10X

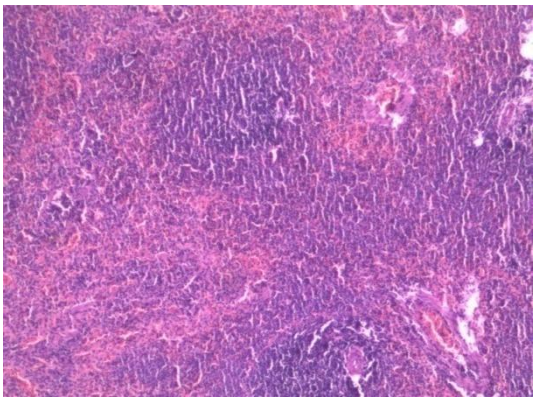


High Power Magnification 40X

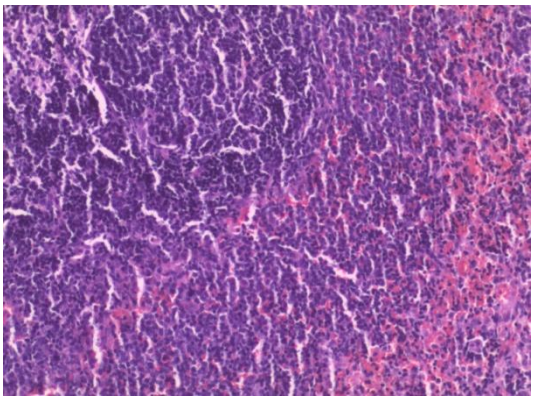


Spleen

Low Power Magnification 10X

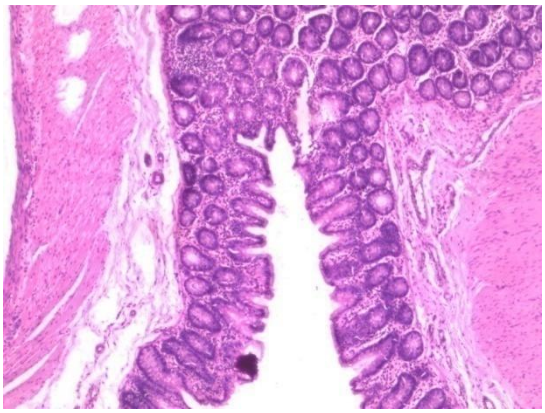


High Power Magnification 40X

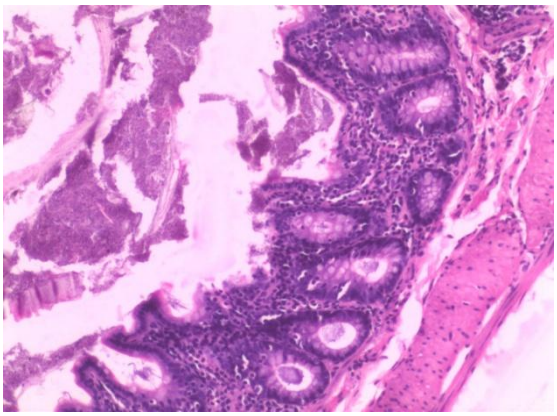


Stomach

Low Power Magnification 10X



High Power Magnification 40X



Methodology

All the vital organs obtained were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

Pathologist report

Kidney

- Appearance of Podocytes and parietal epithelium in the glomeruli appears normal in 3 CM , where as derangement in the cytoarchitecture of parietal epithelium was observed in 3HM
- Proximal and distal convoluted tubule appears normal
- No signs of lesion or inflammation were observed
- No signs of cellular necrosis

Heart

- Cardiac muscle appears continuous with intact nucleus
- Myocardial tissue appears normal with orderly striated heart muscle fibers and a clear nuclear and muscle bands.

Liver

- Marginal infiltration of inflammatory cells were observed in sample belongs to 3HM on periportal region of liver section
- Hepatocellular architecture was normal with no signs of necrosis

Spleen

- Appearance of central artery and marginal sinus are normal
- No abnormalities found in lymph node of both the samples

Stomach

- Light microscopic observation of both the sample reveals normal histology of rat gastric wall composed of mucosa, muscularismucosa, submucosa, muscularispropiria and adventitia.
- No signs of ulceration were observed

Interpretation of Sub-chronic toxicity of *Inji dravagam*

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub chronic toxicity study. There was significant change in the body weight for the control and treatment group throughout the dosing period of 90 days.

Hematogological and biochemical parameters of wistar albino rats exposed to *Inji dravagam* have no significant changes when compared to control group.

Intrepretation of histopathology:

The vital organs such as liver, heart, kidneys, stomach and brain were compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

8. PHARMACOLOGY ACTION:

Table: 20 Effect of *Inji Dravagam* on Volume of gastric juice

Group No.	Body wt. gms	Treatment	Vol. of gastric Juice
I	181.3±1.51	Control (distilled water 2ml/kg)	4.7±0.0
II	182.1±2.04	Negative control(Aspirin 500mg/kg)	5.48±0.20
II	181.5±0.84	Standard drug Ranitidine (20 mg/kg)	1.8±0.28**
III	181.8±1.60	*Inji Dravagam* (1ml/kg)	4.0±0.0*
IV	181.3±1.63	*Inji Dravagam* (2ml/kg)	3.16±0.48**

Effects are statistically significant *P<0.05;**p<0.01 (in comparison with Standard)

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)

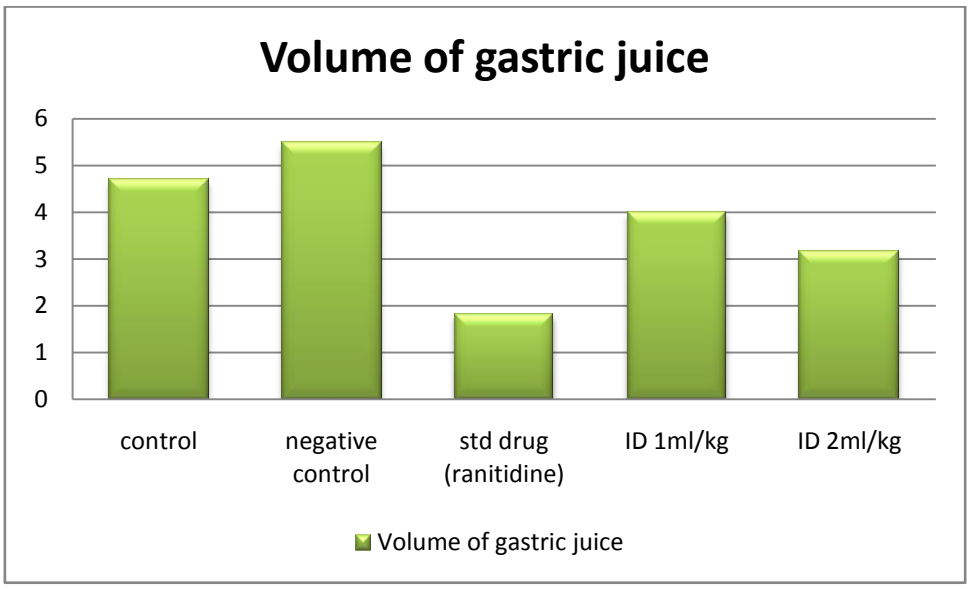


Table: 21 Effect of *Inji Dravagam* on PHin Aspirin induced ulcer model

Group No.	Body wt. gms	Treatment	PH
I	181.3±1.51	Control (distilled water 2ml/kg)	2.16±0.75
II	182.1±2.04	Negative control(Aspirin 500mg/kg)	1.8±0.41
II	181.5±0.84	Standard drug(Ranitidine)	5.16±0.98**
III	181.8±1.60	*Inji Dravagam* (1ml/kg)	3.16±0.41*
IV	181.3±1.63	*Inji Dravagam* (2ml/kg)	4.0±0.00**

Effects are statistically significant *P<0.05;**p<0.01 (in comparison with Standard)

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)

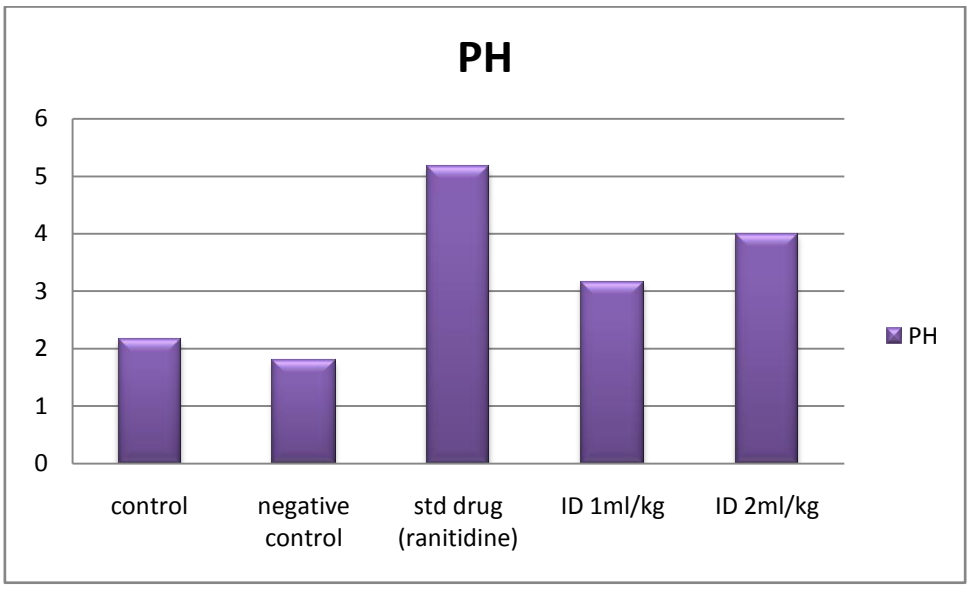
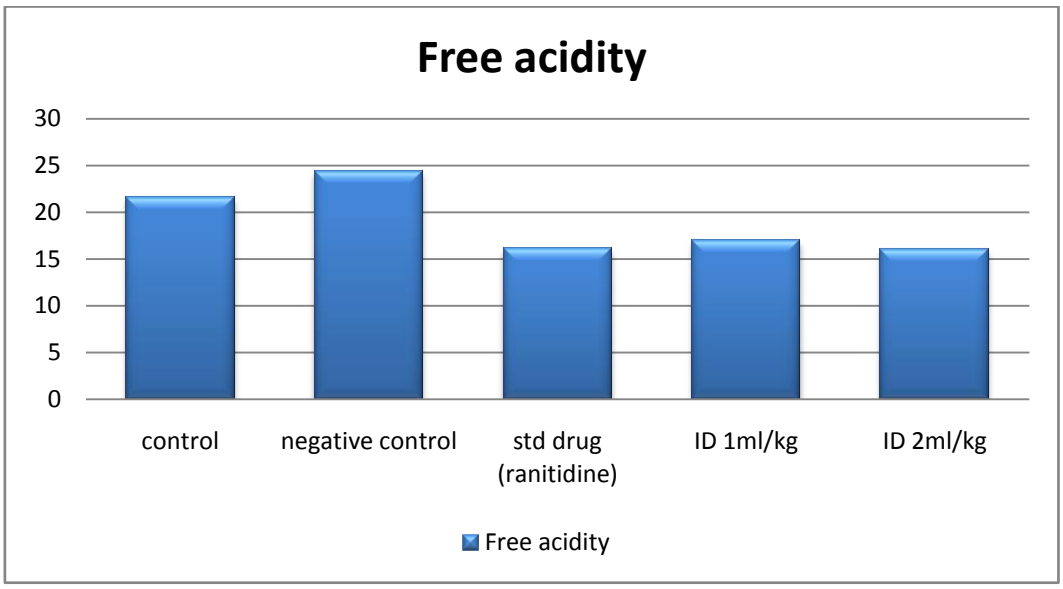


Table: 22 Effect of *Inji Dravagam* on Free Acidity and Total Acid

Group No.	**Body wt. gms**	**Treatment**	**Free acidity**	**Total acidity**
I	181.3±1.5	Control (distilled water 2ml/kg)	21.53 ± 0.62	40.02 ± 1.23
II	182.1±2.0	Negative control(Aspirin 500mg/kg)	24.33 ± 1.20	49.05±0.70
III	181.5±0.84	Standard drug (Ranitidine)	16.11±0.20**	25.63±1.8**
III	181.83±1.6	*Inji Dravagam* (1ml/kg)	16.91±0.38*	27.08±1.06*
IV	181.3±1.63	*Inji Dravagam* (2ml/kg)	16.03±0.21**	27.00±0.57**


Free acidity
30
25
20
15
10
5
0
control
negative control
std drug (ranitidine)
ID 1ml/kg
ID 2ml/kg
Free acidity

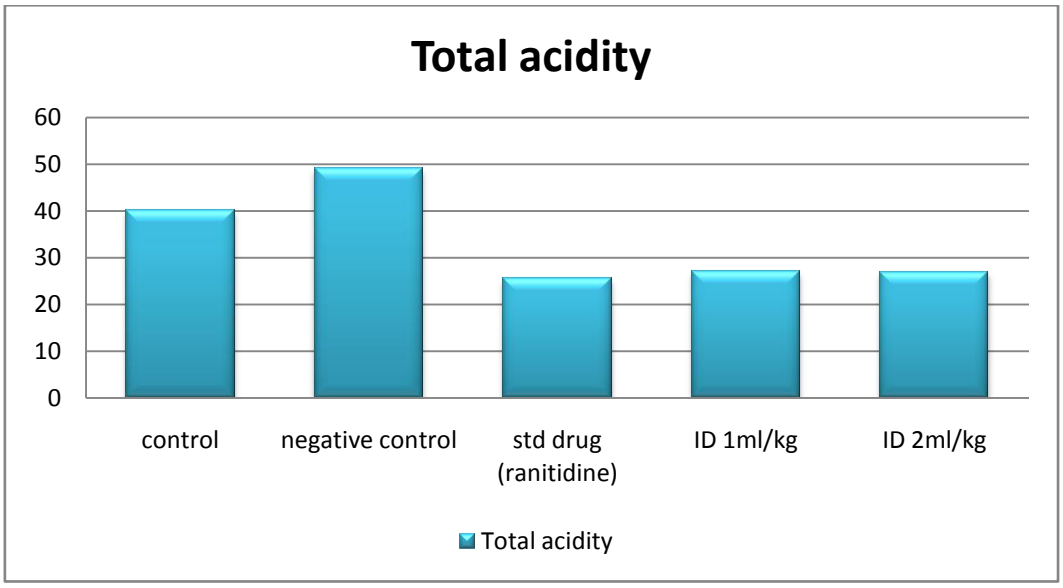

Total acidity
60
50
40
30
20
10
0
control
negative control
std drug (ranitidine)
ID 1ml/kg
ID 2ml/kg
Total acidity

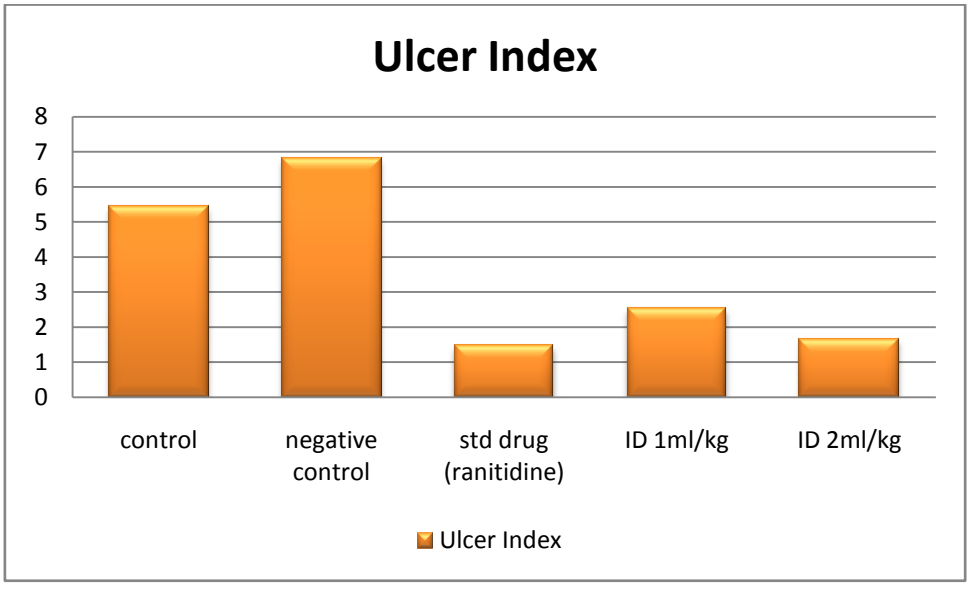
Table: 23 Effect of *Inji Dravagam* on Ulcer index and Percentage of ulcer protection

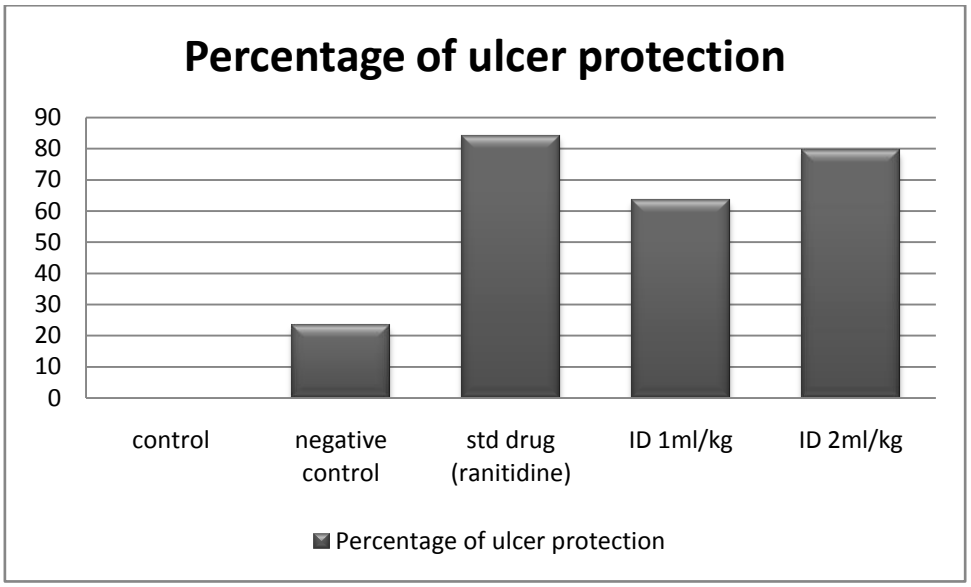
Group No.	Body wt. gms	Treatment	Ulcer Index	Percentage of ulcer protection
I	181.3±1.5	Control (distilled water 2ml/kg)	5.43 ±0.16	-----
II	182.1±2.0	Negative control	6.8±0.58	23.1
II	181.5±0.84	Standard drug(Ranitidine)	1.48 ±0.35	84.03**
III	181.83±1.6	*Inji Dravagam*(1ml/kg)	2.54 ±0.11	63.23
IV	181.3±1.63	*Inji Dravagam*(2ml/kg)	1.66±0.15	79.2*

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)

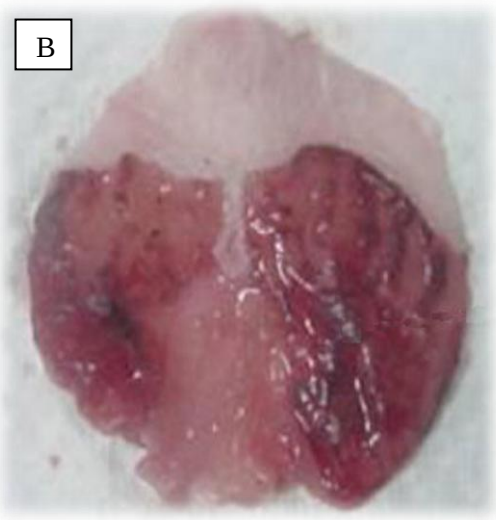
Percentage protection = (Control mean ulcer index – Test mean ulcer index)/Control mean ulcer index ×100

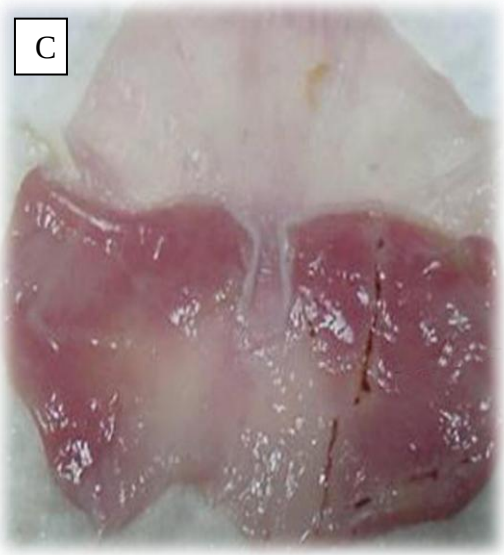
Effects are Statistically significant *P<0.05;**p<0.01 (in comparison with Standard)

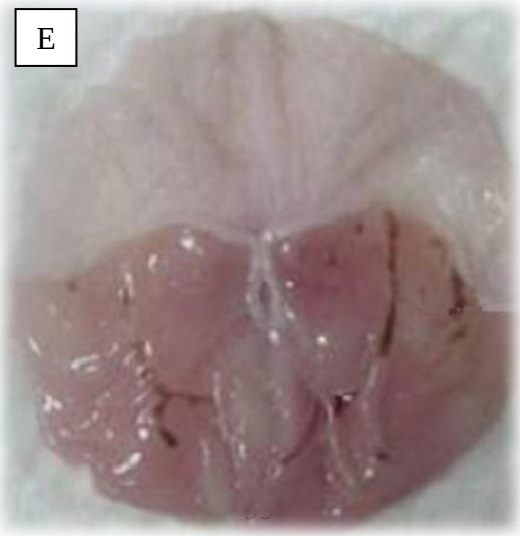



Percentage of ulcer protection
90
80
70
60
50
40
30
20
10
0
control
negative control
std drug (ranitidine)
ID 1ml/kg
ID 2ml/kg
Percentage of ulcer protection

Open excised stomach in Aspirin induced gastric lesions model:







a: Inhibition in gastric lesions at control

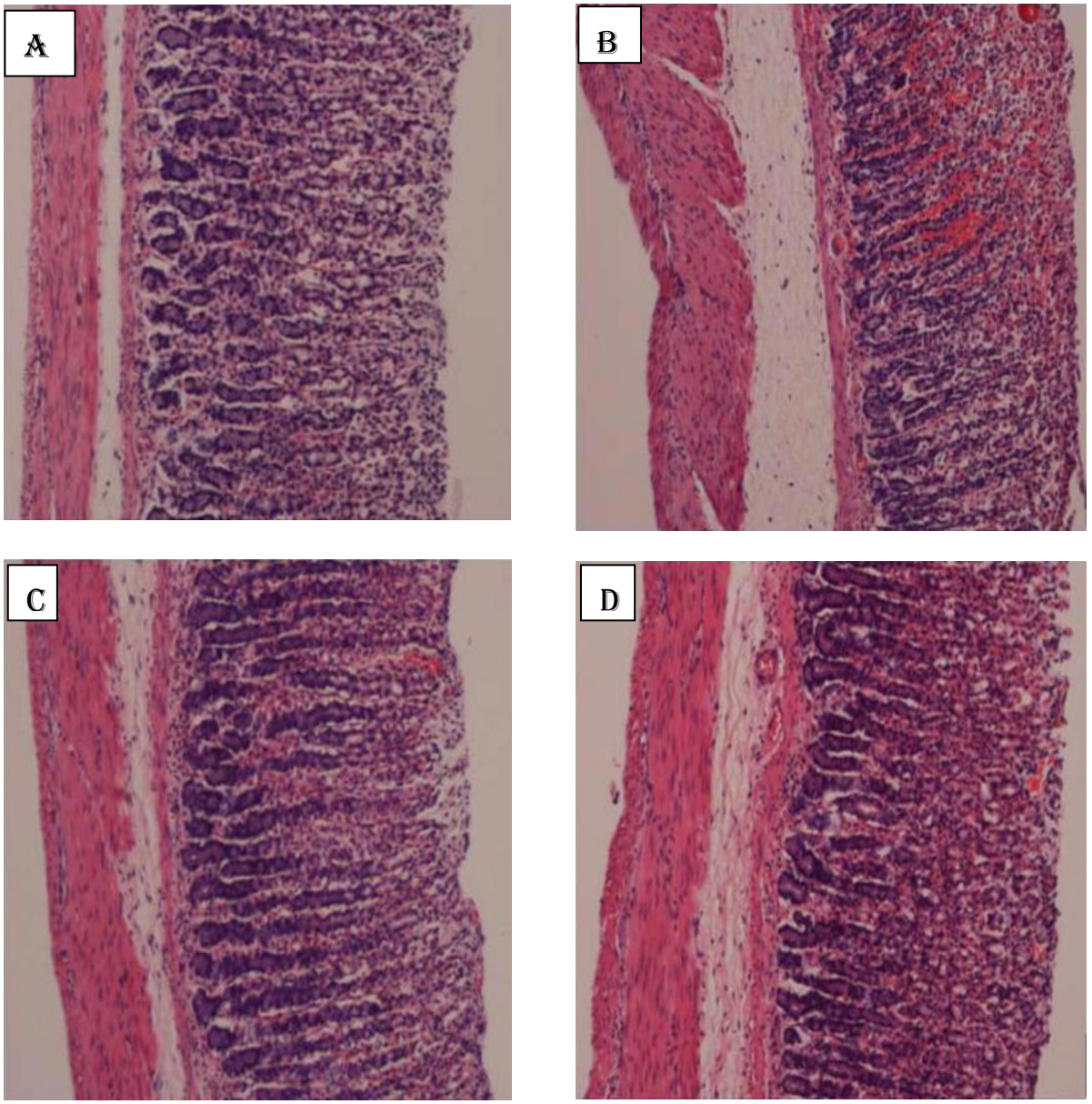
b. Gastric lesions induced by Aspirin (500mg/kg)

c: Absence of gastric lesions in Ranitidine

d: Fraction inhibition of gastric lesions at *Inji Dravagam* (1ml/kg)

e:fractional inhibition of gastric lesions *at Inji Dravagam* (2ml/kg)

Histopathological Examination of Open Excised Stomach in Aspirin Induced Ulcer Method:



a: Inhibition in gastric lesions at control

b. Gastric lesions induced by Aspirin (500mg/kg)

c: Absence of gastric lesions in Ranitidine

d. Fraction inhibition of gastric lesions at *Inji Dravagam*

Results of Anti- ulcer (Hyperacidity) activity of *Inji Dravagam (ID)* in wistar albino rats

Gastric volume

The gastric volume was increased in aspirin induced group when compared to control group. Administration of ID and ranitidine showed a significant ($p<0.01$) decrease in gastric volume level, when compared to negative control.

pH.

The pH level was decreased ($p<0.01$) in the aspirin induced method, when compared to control group. Administration of ID and ranitidine showed a significant ($p<0.01$) increase in pH level, when compared to negative group animals. Results were showing Table 21.

Free and total acidity

The free acidity (mEq/l/100g) was increased ($p<0.01$) in the aspirin induced animals, when compared to control group. Administration of ID and ranitidine showed a significant ($p<0.01$) decrease in free acidity, when compared to negative control.

Total acidity (mEq/l/100g) was increased ($p<0.01$) in aspirin induced model, when compared to control group. Administration of ID and ranitidine showed a significant ($p<0.01$) decrease in total acidity, when compared to negative control.

Conclusion

Inji Dravagam exhibited anti ulcer activity in aspirin induced ulcer model for screening anti ulcer drugs. The percentage of inhibition of ulcer was 84.03%, 63.23%, 79.2% produced by the treatment of standard drug Ranitidine, *InjiDravagam* (ID) at the dose level of 1ml/kg and 2ml/kg respectively. In case of vehicle control, aspirin induced rats showed increase in acid secretion, which in turn caused increase in gastric volume,

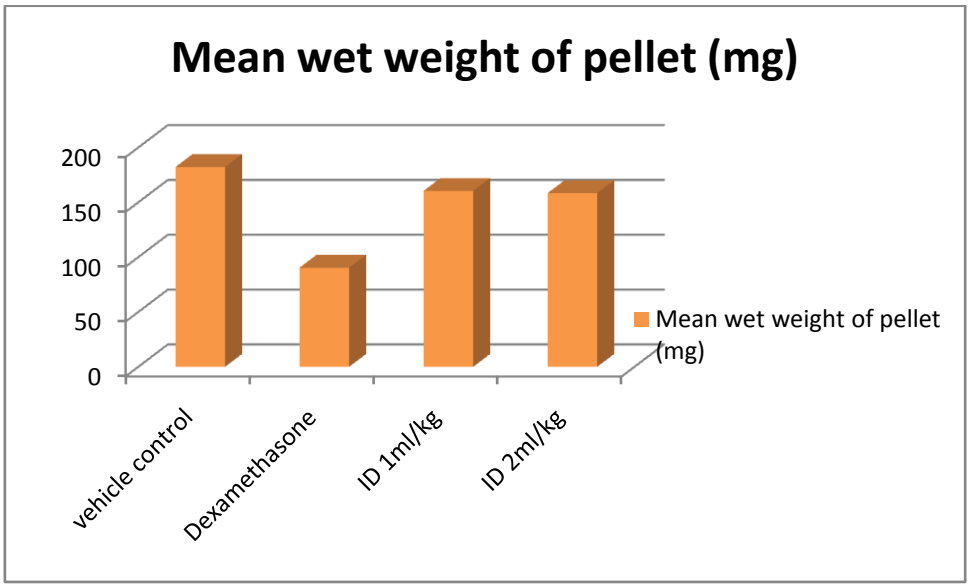
low pH, increased free and total acidity resulting in increase of ulcer index.

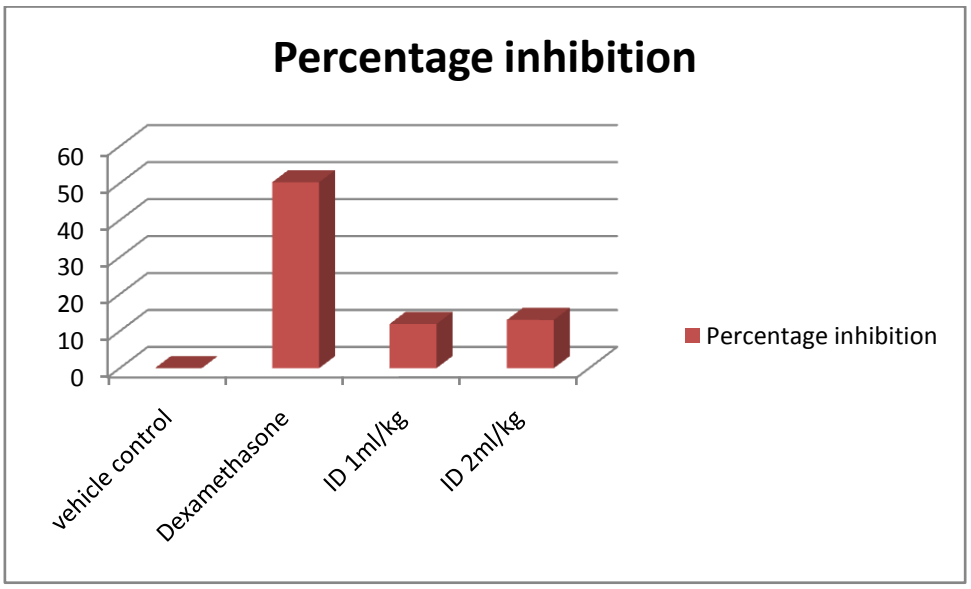
Inji Dravagam (ID) when administered at dose level of (1ml/kg and 2ml/kg) produced a reduction in the gastric fluid volume, free acidity, total acidity and ulcer index significantly in comparison with control group.

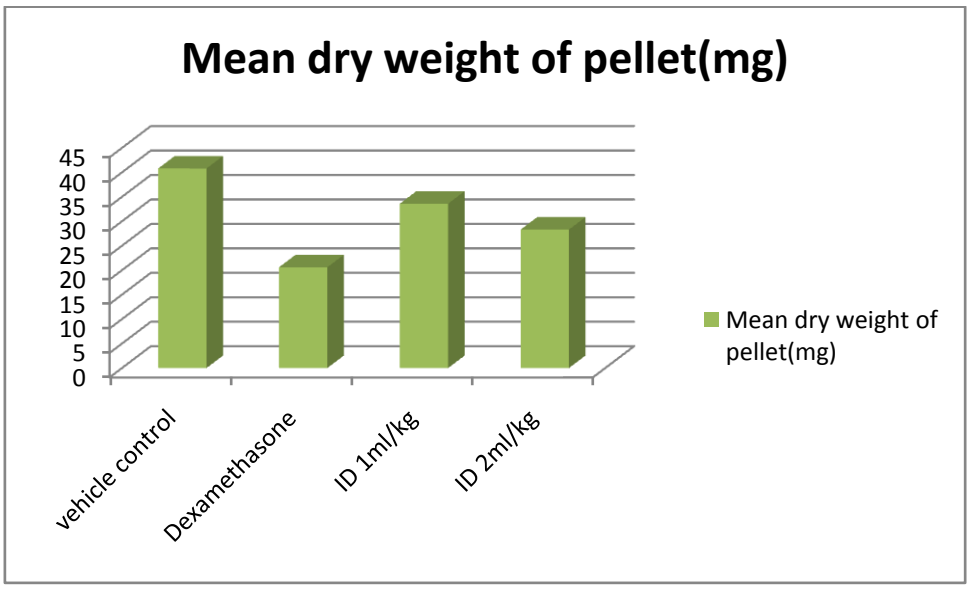
Hence, based on the results, it can be concluded that the *Inji Dravagam*, significantly $p<0.05$ and $p<0.01$,decreased the ulceration in Aspirin induced ulcer model in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 1ml/kg and 2ml/kg respectively. The result was represented in table 23.

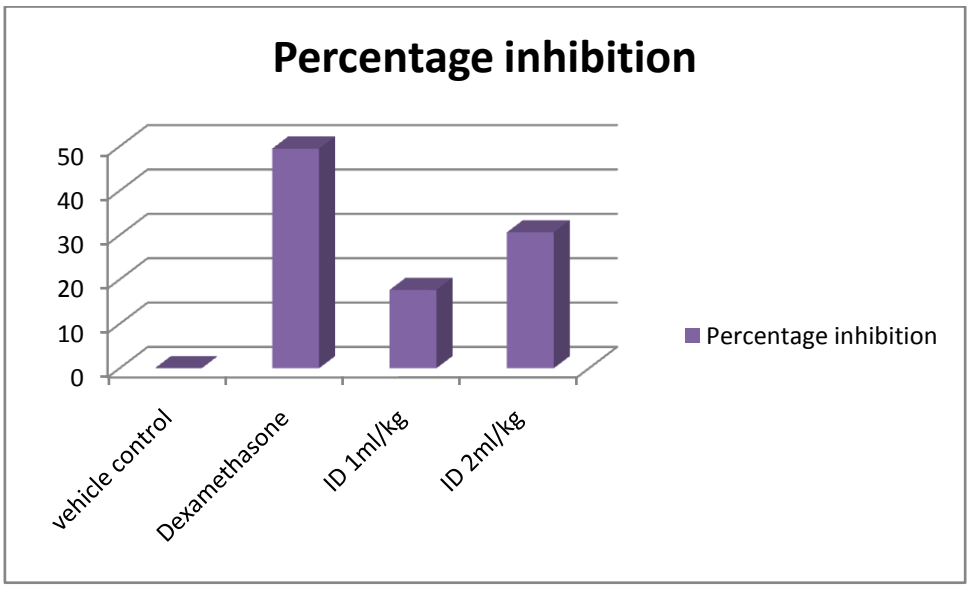
Table :24 The results of anti- inflammatory activity by Cotton pellet granuloma method of *Inji Dravagam*

Groups	Treatment	Mean wet weight of pellet(mg)	Percentage inhibition	Mean dry weight of pellet(mg)	Percentage inhibition
I	Vehicle control	182.2	0	40.8	0
II	Dexamethasone(0.5mg/kg)	90.4	50.3	20.6	49.50
III	*InjiDravagam*(1ml/kg)	160.4*	13.06	33.6	17.64
IV	*InjiDravagam*(2ml/kg)	158.4*	11.96	28.3	30.63




Percentage inhibition
60
50
40
30
20
10
0
vehicle control
Dexamethasone
ID 1ml/kg
ID 2ml/kg
Percentage inhibition


Mean dry weight of pellet(mg)
45
40
35
30
25
20
15
10
5
0
vehicle control
Dexamethasone
ID 1ml/kg
ID 2ml/kg
Mean dry weight of pellet(mg)

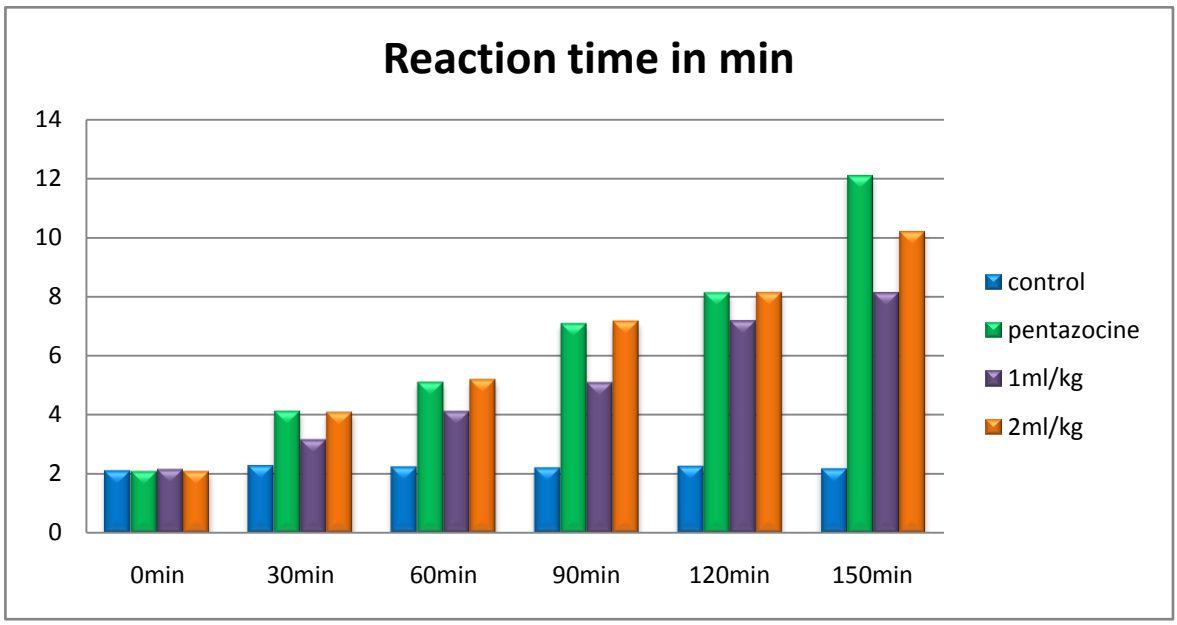


RESULTS

The results indicate that *Inji dravagam* at the dose level of 1ml/kg and 2ml/kg produced a decrease in wet granuloma weight 160.4 (11.96% inhibition) and 158.4 (13.06% inhibition) respectively when compared to control. Similarly there was a significant decrease in dry granuloma weight 33.6(17.64% inhibition) and 28.3(30.68% inhibition) respectively compared to control. Among the two doses 2 ml/kg showed slightly lower reduced weight of granuloma than standard drug which was showed in table 24. Thus it was concluded that administration of *Inji Dravagam* at the dose of 2 ml/kg exhibited significant ($p<0.05$) anti-inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

Table : 25 Analgesic activity of *Inji Dravagam* by eddys hot plate method.

Groups	Treament	Reaction time in min					
		0min	**30min**	**60min**	**90min**	**120min**	**150min**
I	Control	**2.08±0.07**	**2.25±0.10**	**2.21±0.11**	**2.18±0.13**	**2.23±0.10**	**2.15±0.08**
II	Pentazocine (5mg/kg)	**2.09±0.04**	**4.13±0.07**	**5.11±0.07**	**7.08±0.13****	**8.12±0.10****	**12.08±0.07****
III	ID (1ml/kg)	**2.16±0.05**	**3.15±0.08**	**4.11±0.06**	**5.08±0.08**	**7.16±0.13**	**8.10±0.07***
IV	ID(2ml/kg).	**2.09±0.04**	**4.09±0.04**	**5.20±0.08**	**7.16±0.13****	**8.12±0.10****	**10.18±0.08****



n=6 ,values are expressed as mean±SEM P<0.05 when compared with control.

Analgesic activity of *Inji Dravagam* by eddy's hot plate method.

The results were analyzed by ANOVA followed by Dunnet's test (p-value <0.05 was taken as significant).

Analgesic activity of *Inji dravagam* in swiss albino mice

Analgesic activity was carried out by Eddy's Hot plate method. Analgesic effect lasted for a period of 120 min was found to be possess significant ($p<0.01$) analgesic activity at the dose level of 2ml/kg by increase in reaction time (Increase threshold potential of pain). *Inji Dravagam* 1ml/kg showed moderate activity when compared to standard drug pentazocine, whereas *Inji Dravagam* at 2ml/kg showed results more similar to that of pentazocine (5 mg/kg). From these results it is obvious that *Inji Dravagam* has significant analgesic activity

DISCUSSION

DISCUSSION

The drug ***Inji dravagam*** was selected to study the anti-ulcer, Anti-inflammatory and Analgesic activity. The trial drug was selected from "yaakopu vaithiya chinthamani"

The preclinical study substantiated the literary evidence of *Inji dravagam* in the management of Gastrointestinal disorders.

Bio-chemical analysis:

Biochemical analysis of the drug *Inji dravagam* reveals that the presence of Chloride, Calcium , Ammonium and alkaloids.

Toxicological studies:

This study reveals that no significant toxic effect of the drug *Inji dravagam* upto the higher dose level 10ml/kg in acute oral toxicity and also sub acute toxicity and sub chronic toxicity has no toxic effects from the results. Therefore the drug *Inji dravagam* can be classified under the category of drug with non-toxic.

Pharmacological studies:

In the Pharmacological study the experimental data showed that Inji dravagam has Anti-ulcer, Anti-inflammatory and Analgesic activity and the results are as follows,

Anti-ulcer

Inji Dravagam was found to possess remarkable ulcer protective properties and almost exhibited similar effects as that of ranitidine. Hence, based on the results, it can be concluded that the *Inji Dravagam*, significantly decreased the ulceration in Aspirin induced gastric ulcer in rats which suggest a direct ulcer protective effect on the gastric mucosa.

activity when compared to standard drug pentazocine, whereas *Inji Dravagam* showed results more similar to that of pentazocine (5 mg/kg). From these results it is obvious that *Inji Dravagam* has significant analgesic activity.

Anti-inflammatory

Inji dravagam exhibited significant anti-inflammatory activity when compare to the control

SUMMARY

SUMMARY

- The literary evidence of the drug *Inji Dravagam* strongly support that it possesses anti-ulcer activity, Anti-inflammatory activity and analgesic activity for that purpose it has been selected for this study.

- The qualitative chemical analysis was done at Biochemistry lab, NIS. chemical analysis of the drug *Inji Dravagam* reveals that the presence of calcium,, Chloride, ammonium and Alkaloid.

- Preclinical evaluation (acute, sub-acute and sub-chronic toxicity study) of the drug was carried out as per OECD guideline acute, and Sub-acute was carried out in kk coll of pharmacy and sub chronic toxicity study were carried out in pharmacological lab and animal house, NIS, Chennai. This study reveals no significant toxic effect of the *Inji Dravagam* upto the higher dose level 10ml/kg used in this study.

- Pharmacological study (Anti-ulcer activity, Anti-inflammatory activity and analgesic activity) of the drug was carried out as per OECD guideline in KK College of Pharmacy Gerubambakkam. In the pharmacological studies, the drug *Inji Dravagam* exhibits significant Anti-ulcer, Anti- inflammatory activity and analgesic.

CONCLUSION

CONCLUSION

From the literature evidence, Physico chemical analysis, Bio chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Inji Drvagam* has Anti-ulcer, anti-inflammatory and analgesic activity. It is concluded that the drug *Inji Dravagam* can be used in the management of Gunmam (peptic ulcer) and the related Gastro intestinal disorder.

BIBLIOGRAPHY

BIBILIOGRAPHY

1. S.P.Ramachandhiran, yaakopu vaithiya chinthamani-700 Third edition-1992, published by by Indian medicine and homeopathy,

2. Samuvel, maarunthu sei iyalum kalaium, first edition, published by Dr.A.Sundarajan.

3. sarakku suthi muraigal, published by Indian medicine and homeopathy- 2008

4. S.murugesamuthaliyar, Gunapadam mooligai vaguppu, ninth edition, published by Indian medicine and homeopathy, volume -1.

5. Shanmugavel, noigalukku Siddha parigarum, fourth edition, published by Indian medicine and homeopathy, volume -1.

6. [7].Kannusamipillai, seegecharathina thepam, first edition, published by by Indian medicine and homeopathy.

8. vaithiya sindhamani, first edition, published by Indian medicine and homeopathy page no 159.

9. S.P.Ramachandhiran, yaakopu vaithiya chinthamani-700 Third edition-1992, published by by Indian medicine and homeopathy,

10.P.S.Kuppusamy, annupogavaithiya brammaragasiyam, second edition, published by by Indian medicine and homeopathy,

11. V.R.Madhavan, Agathiar vaithiyakaviyam1500, published by by Indian medicine and homeopathy,

12. Dr.J.Ramachandran, Herbs of Siddha medicine, published by Jayanthi Raamachandran, volume I &II, first edition: January, 2008

13. MDidea Extracts professional www.mdiea.com sep 28th 2015

14. Gaurav Kumar, L. Karthik, K. V. Bhaskara Rao, Gaurav Kumar et al. / Journal of Pharmacy Research 2011,

15. Dr.k.m. Nandkarni, Indian metriya medica, volume-1, published by ramadas bhatkal

16. A. Anosike, Onyechi Obidoa, African Journal of Biochemistry Research Vol.3 (12), December, 2009, ISSN 1996-0778 © 2007, www.academicjournals.org/ajbr

17. Rajesh Kumar Mishra, international journal of pharmaceutical and chemical sciences.

18. A. Anosike, African Journal of Biochemistry Research Vol.3 (12), pp. 379-384, December, 2009 online at http://www.academicjournals.org/ajbr

19.A.K.Gupta, qualitative standards of Indian medicinal plant volume-3 published by Indian council of medical research V.Ramalingamswami

20. Nagalakshmi, S.; Shankaracharya, Journal of Food Science and Technology (Mysore) 2000 Vol. 37 No. 3 INSS NO-0022-1155

21.Mohammad Hossein Boskabady, BioMed Research International Volume 2014 (2014), Article ID 569087, http://dx.doi.org/10.1155/2014/569087

22. S. N. Dwivedi, R. P. Mishra and Sangeeta Alava international journal of pharmacy & life sciences, ISSN: 0976-7126,

24. BabyChauhan, American journal of pharmetech research 2012, ISSN 2249-3387,www.ajptr.com.

(chemical)Ranjan bairwa, Pharmacognosy Review, 2012, Volume : 6, Issue : 11,

25.Saleh Alqasoumi, African Journal of Pharmacy and Pharmacology Vol. 5(5), pp. 572-576, May 2011 online http://www.academicjournals.org/ajpp

26.The Siddha formulory of india, part-2, first edition,

27. The Siddha pharmacopoeia of india, part-1 voll-2 first edition.

28.D.R Lohar; Protocol for Testing; Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory For Indian Medicines Publication, Ghaziabad,

29.OECD. Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Organization for Economic Cooperation and Development, Paris, France; 2002

30.OECD. Repeated dose oral toxicity test method. In: OECD Guidelines for testing of chemicals, No. 407. Organization for Economic Cooperation and Development, Paris, France; 2008

31.Organization of Economic Co-operation and Development (OECD) *The OECD Guideline for Testing of Chemicals: 408 Subchronic Oral Toxicity-Rodent: 90-Day Study.* Paris, France: OECD; 1998.

ANNEXURE

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Inji Dravagam"** (Internal) taken up for Post Graduation Dissertation studies by **Dr.S.Dinesh**, M.D.(S), II year, Department of Gunapadam, 2015, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Zingiber officinale Rosc. (Zingiberaceae), Fresh rhizome.

Carum copticum Benth. & Hook. f. (Apiaceae), Fruit

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB1982015

Date: 14-8-2015

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

PROJECT COMPLETION CERTIFICATE

This is to certify that the project entitled **A Safety and Pharmacological Profile of Inji Dravagam** has been approved by the IAEC committee of our institution. Dr. S. Dinesh has completed the project work under the guidance of Mrs. C. Senthil Kumari, Associate Professor, Dept., of Pharmacology, K.K. College of Pharmacy, Chennai.

(Mrs. C. Senthil Kumari)
Associate Professor
Project Guide

(Dr. A. Meena)
Principal

K.K. COLLEGE OF PHARMACY
CHENNAI
600 122

CERTIFICATE

This is certify that the project title..... SAFETY PROFILE OF "INJI DRAVAGAM" (Approval no: NIS/IAEC-I/2016/03). **has been approved by the IAEC.** (12 Male and 12 Female Wistal albino rats)

Name of Chairman/Member Secretary IAEC nominee:

Dr. B.R. SENTHILKUMAR

Signature with date

24. Feb 2016

Chairman/Member Secretary of IAEC:

Name of CPCSEA

K. NAGARAJAN

17/02/2016

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

K.K. COLLEGE OF PHARMACY

(Approved by AICTE, PCI & Government of Tamilnadu and
Affiliated to The Tamilnadu Dr. MGR Medical University)

1/161, Sankaralinganar Road, • Gerugambakkam, • Chennai - 600128

Phone : (044) 32546162, Tele/Fax : 23821272

Ref: 4524/KKCP/2015 **Date: 10.08.2015**

APPROVAL CERTIFICATE

This is to certify that the project title "A *Safety and pharmacological profile of "**INJI DRAVAGAM**"* has been approved by IAEC and the details are furnished under

Project Code	Name of the species	Breakup sexwise	Weight	Number proposed	Number approved
KKCP/2015/028	Swiss albino mice	12 Male + 12 female	25-30 gms	24	24
	Wistar Albino rat	40 Male + 46 female	130–140gms	90	86
Albino mice- Twenty four only; Albino rats – eighty six only					

Chairman IAEC

(Prof. A. Meena)

CPCSEA Nominee

(Dr. C. Kathirvelan)

Veterinary Officer

(V. VAIDHYALINGAM)

Members

Dr K Sadasivam Pillai

SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY
INDIAN INSTITUTE OF TECHNOLOGY, MADRAS
Chennai - 600 036. INDIA

CERTIFICATE

This is to certify that Herbal Drug **Inji Dravagam** formulated by **Dr.S.Dinesh,** III year M.D(S), Department of GUNAPADAM, National Institute of Siddha, Chennai-47. Was analysed (qualitative/quantitative) by, GC-MS and ICPOES methods at SAIF, IITM, Chennai-36, during March 2016.

[DR.R.MURUGESAN]

Dr. R. Murugesan
Senior Scientific Officer
SAIF, IIT, Madras, Chennai-36.

Phone : 91-44-2257 4935 Fax : 91-44-2257 0545, 2257 0509
e-mail : saif@iitm.ac.in http://www.saif.iitm.ac.in

The Tamil Nadu Dr.M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ S. DINESH

for participating as ~~Resource~~ ~~Person~~ / Delegate in the Fourteenth Workshop on

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 5th to 9th May 2014.

Dr. N. KABILAN M.D. (Siddha)
Reader, Dept. of Siddha

Dr. JHANSI CHARLES, M.D.
Registrar

Prof. Dr. D. SHANTHARAM, M.D., D.Diab.,
Vice-Chancellor

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ACKNOWLEDGEMENT

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- I express my sincere thanks to **Dr.S.Visweswaran M.D(s),** Lecturer, Department of Gunapadam, NIS, Chennai-47, for his suggestions, hopeful support and encouragement of my whole study.

- I express my sincere thanks to **Dr.S.Sivakkumar M.D(s),** Lecturer, Department of Gunapadam, NIS, chennai-47 for his suggestions, hopeful support and encouragement of my whole study.

- I express my sincere thanks to **Dr.A.Mariappan M.D(s),** Lecturer, Department, of Gunapadam NIS,Chennai-47, for his suggestions, hopeful support and encouragement of my whole study.

- I express my sincere thanks to **Dr.V.Suba M.Pharm, P.hD.,** Assistant Professor in Pharmacology, NIS, Chennai-47, for her suggestions in the pharmacological study.

- I express my grateful thanks to **Dr..J.Rani B.V.sc,** veterinarian,(Late) NIS, for her healthy support to me and my test animals throughout my studies

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- I express my sincere thanks to **Dr.D.Aravind M.D(s), M.Sc.,** Assistant Professor, Medicinal Botany, NIS, chennai-47.

- I express my grateful thanks to **Dr.Senthil kumari.,M.Pharm Ph.D,** KK college of pharmacy, Gerugambakkam, Chennai-97, for his assistance in the pharmacological study.

- I express my sincere thanks to **Mr.M.Subramanian M.Sc.,** (statistics) Senior Research Officer, National Institute of Siddha, Chennai-47.

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